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Earthworm giant nerve fibers: an electrophysiological and behavioral study

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Earthworm giant nerve fibers: An electrophysiological and behavioral study

by

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PREFACE

The selection of a scientific problem in neurobiology involves choosing between two distinct sets of objectives. The investigator may concern himself with characterizing mechanisms underlying nerve cell and synaptic functioning, or, on the other hand, he may study neural functioning in respect to its role at higher integrative levels, leading hopefully to a fuller understanding of the neural substrates of behavior in the organism.

In the case of mechanism-level studies, powerful research tools have been developed and applied in neurobiology over the last few decades. For example, electron microscopy has been employed in examining in detail the anatomical correlates of neural activity, and coupled with more recently developed intracellular staining and recording techniques using glass microelectrodes, has provided insight into connectivity patterns in many different animal preparations. Intracellular microelectrode studies have also elucidated many of the chemical and electrical processes involved in specific nerve activities. In fact, the primary focus of interest in neurobiology for some time has been on mechanism-level research. Tremendous progress has been made in this regard, and we now understand many of the cellular and subcellular processes which provide the basis for neural functioning.

In the case of studies concerning the neural substrates of

behavior, progress has been slower and sporadic (for an excellent treatment of recent research and a discussion of problems in this area see Fentress, 1976). Many efforts have been made to use the tools of mechanism-level research to investigate neural correlates of behavior, but the disruptive and destructive consequences of invasive techniques have generally precluded recording of neural functioning within a reasonably normal behavioral context. Obviously, minimally disruptive or non-invasive methods are a prerequisite to this type of investigation. The papers herein represent an attempt to examine neural and effector correlates of escape behavior in intact, freely moving earthworms. As is frequently true in scientific research, the apparent novelty of the electrophysiological methods employed in these studies actually derives from largely overlooked observations of earlier investigators (Rushton and Barlow, 1943) which remained undeveloped due to the lack of an appropriate technology.

The ideas and technology for noninvasive recording of bioelectric and behavioral phenomena have been developed under the creative supervision of Dr. Charles Drewes, and are the result of several stages of refinement. As these studies have progressed, both the appropriateness of the earthworm as an experimental subject and the quality and quantity of information which can be obtained using noninvasive recording techniques have become

more apparent. Current studies (to be reported in later papers) have progressed to include characterization of *in vivo* properties of regenerating giant fibers and recording specific neural and efferent activities from undisturbed animals in the field. The potential for further development of these recording techniques and their incorporation into an automated system for acquisition and analysis of bioelectric and behavioral data is promising. These techniques should be particularly useful in studies of developmental aspects of behavior, behavioral plasticity, as well as daily and seasonal variations in neural functioning and behavior. It is hoped that the studies herein represent an initial and worthwhile contribution to the challenging interface of neurobiology and behavioral science.

This dissertation is written in the scientific manuscript format. The balance of the text consists of four scientific papers, one already published (Drewes, Landa, and McFall, 1978), and three to be submitted shortly for publication.

PART I. GIANT NERVE FIBER ACTIVITY IN INTACT, FREELY MOVING EARTHWORMS

INTRODUCTION

Many animals escape from predators or noxious stimuli by means of specialized and stereotyped locomotory responses. In some arthropods and annelids these responses are very rapid, being mediated by giant nerve fiber activity that is well-suited for electrophysiological recording at the level of individual nerve cells (Wiersma, 1947; Nicol, 1948; Kennedy, 1966; Zucker, 1972a, b; Parnas and Dagan, 1971). The recording of such activity, however, usually requires procedures (e.g. immobilization, dissection, and exposure to physiological saline solutions) which are invasive and potentially disruptive to both the neural functioning and behavior being studied.

In some arthropods progress has been made in developing minimally invasive methods (e.g. implanted and tethered electrodes) for recording single neural unit activity and for correlating this activity with locomotory or escape behavior (Stout, 1971; Delcomyn, 1976; Schrameck, 1970; Wine and Krasne, 1972). Also, in the marine annelid Branchiomma vesiculosum (Polychaeta), Krasne (1965) has noninvasively recorded giant axon and muscle responses to tactile stimulation using a pair of wire electrodes threaded into the animal's tube. The possibility of recording neural activity

from undissected earthworms was first demonstrated by Rushton and Barlow (1943). Their brief study was restricted to recording, through the skin of restrained earthworms, the medial giant fiber response to an electrical shock. They also limited their recording to a single, central location on the worm.

In this study we have developed noninvasive methods for recording giant nerve fiber activity at two selected sites from unrestrained earthworms. This has permitted study of various giant fiber parameters in situ as well as correlation of giant fiber activity with locomotory and escape behavior.

MATERIALS AND METHODS

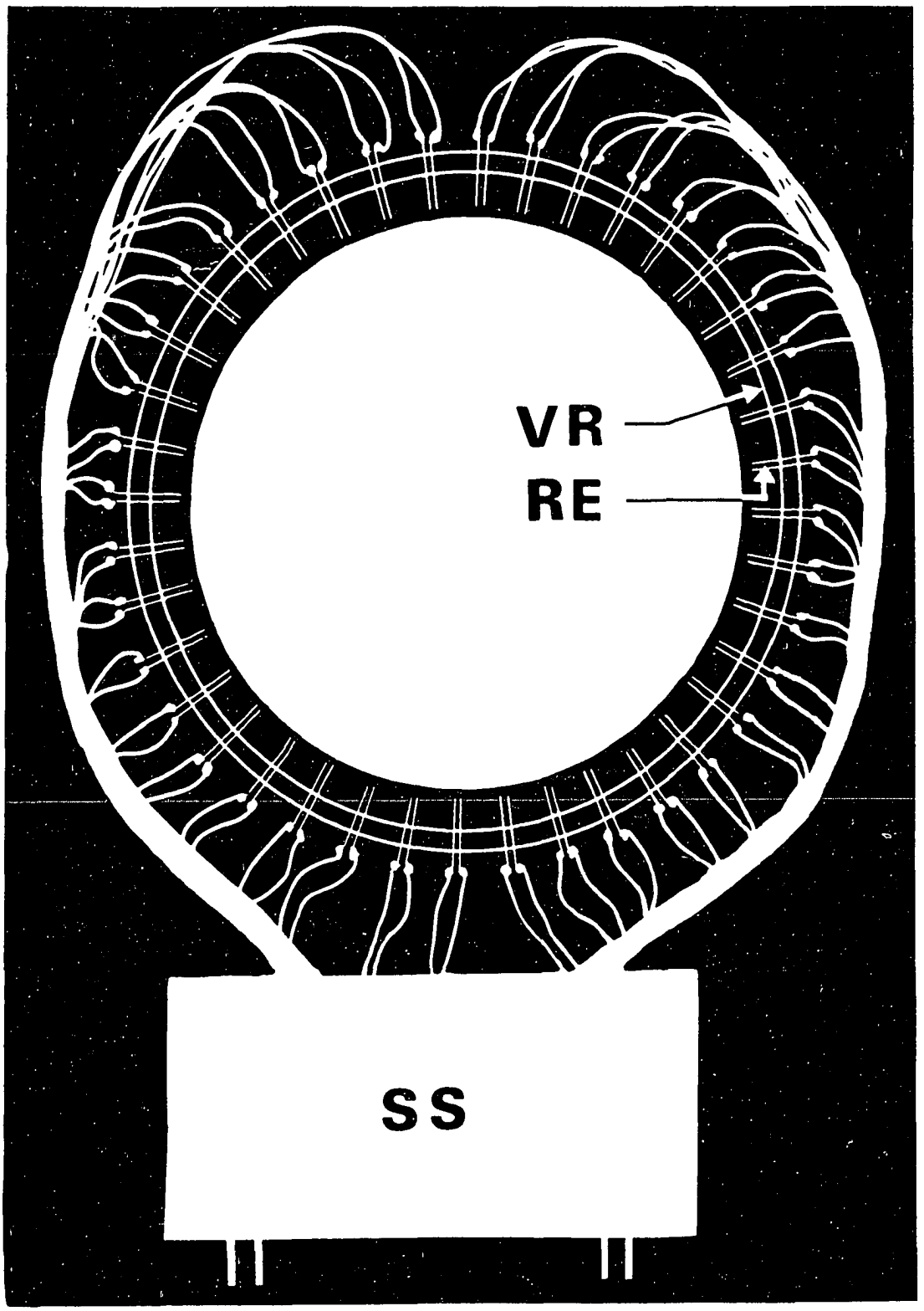
Recordings from Intact Animals

Mature earthworms, Lumbricus terrestris L., were obtained from Mogul Corp. (Oshkosh, Wis., U.S.A.) and maintained at 15 °C in Buss Bed-ding (Buss Mfg., Lanark, Ill., U.S.A.). All experiments were carried out at 21-22 °C.

The experimental apparatus for recording giant fiber activity from intact, freely moving animals is shown in Fig. 1. Two concentric vinyl rings (78.1 and 81.9 cm circumferences; 5 cm high) formed a circular track (mean circumference 80.8 cm). The rings rested on a radiating array of 40 pairs of silver wire electrodes (0.405 mm diameter), each pair being spaced 20 mm apart. A nearly optimal signal : noise ratio for giant fiber spikes was obtained when the electrodes in a pair were spaced 3 mm apart. The electrodes rested on the track surface which consisted of wet-dry sandpaper (fine grit). This surface provided traction for the animal and was moistened with distilled water to prevent desiccation of the animal. The entire apparatus was mounted on sponge rubber to absorb extraneous vibrations.

Recording electrode pairs (numbered 1-40) were selected via a two-level switching system which permitted us to simultaneously record from two adjacent sites as the animal moved around the track. The first level of switching consisted of eight 10-position

Fig. 1. Top view of apparatus for recording activity of giant fibers in intact earthworms. Animals were placed in between two concentric vinyl rings (VR) and were allowed to move freely over a radiating array of 40 pairs of silver-wire recording electrodes (RE). Giant fiber activity could be recorded from any two sites selected by the switching system (SS). Signals were led from the switching system to two pre-amplifiers. (Drewes, Landa, and McFall, 1978)


VR
RE
SS

single-contact rotary switches allowing selection of two even-numbered and two odd-numbered electrode pairs. The second level consisted of two double-pole, double-throw toggle switches allowing the final selection of one odd-numbered and one even-numbered pair of electrodes. Signals from each toggle switch were recorded differentially and amplified with a Grass P-15 preamplifier. Outputs from the two preamplifiers were led into separate channels of a Tektronix 5103N storage oscilloscope. All recording apparatus, except the oscilloscope, were contained in an electrically shielded cage. Neural spikes were easily identifiable on the basis of duration (< 1.5 ms) and consistent amplitude during repetitive discharge. Optimal signal : noise ratios (up to 8:1) for giant fiber activity were obtained with a high-frequency filter setting of 1 or 3 kHz and a low-frequency filter setting at 300 Hz. Stored records of giant fiber activity were photographed either as recurrent, superimposed sweeps (e.g. Fig. 5) or as single sweeps triggered by the initial phase of the first giant fiber spike (e.g. Figs. 2-4).

Tactile stimulation was applied (> 5 min intervals between tests) by touching the animal with a hair mounted on a glass rod. Quantitative control of the stimulus strength was not necessary for the purposes of this study; after a few trials we found that with subjective control of stimulus strength we could elicit predictable giant fiber responses. No diminution of giant fiber

responsiveness occurred during the usual testing period (4 h) and it appeared that testing could have continued for a considerably longer time.

Recordings from Dissected Animals

In some experiments it was necessary to correlate giant fiber activity in undissected and dissected portions of the same animal. Dissected preparations were obtained by pinning down approximately 20 segments in the middle of the worm, exposing the ventral nerve cord in this region, and adding several drops of physiological saline (Drewes and Pax, 1974a). Recordings of ventral nerve cord activity were obtained at the air-saline interface using a pair of silver-wire hook electrodes. Recordings were simultaneously obtained from the undissected posterior end of the same animal by looping the body over a similar pair of electrodes. Activity from each recording site was amplified and displayed as described in the previous section.

RESULTS

Electrical activity was recorded from ten intact and freely moving animals in both the presence and absence of experimenter-applied stimulation. Two types of spiking activity were clearly distinguishable on the basis of spike conduction rate and direction of spike propagation. Of 314 spikes recorded, approximately one half were conducted in an anterior-posterior direction at a grand mean rate of 32.2 m/s ± 1.0 S.E.M. (Table 1). The other spikes were conducted in a posterior-anterior direction at a grand mean rate of 12.6 m/s ± 0.3 S.E.M. These two rates are within the broad ranges given for the medial and lateral giant fibers, respectively, of isolated nerve cords in earthworms (Bullock, 1945; Bullock and Horridge, 1965). Also the opposite directions of spike conduction correspond to those of medial and lateral giant fibers of dissected preparations (Stough, 1930; Rushton, 1946; Bullock, 1945). This suggests that the activity we have recorded somehow involves the medial and lateral giant fibers.

Medial Giant Fiber Activity

Activity evoked by tactile stimulation of the anterior few segments of intact animals was always conducted in an anterior-posterior direction at a conduction rate corresponding to that of the medial giant fiber (MGF). The activity had a complex waveform,

Table 1. Conduction rates of MGF and LGF spikes in intact earthworms

Animal no.	MGF			LGF		
	Mean conduction rate (m/s)	S.D. (m/s)	No. of measure-ments	Mean conduction rate (m/s)	S.D. (m/s)	No. of measure-ments
1	38.1	5.1	24	13.7	2.0	21
2	31.4	6.8	17	13.4	1.8	13
3	29.2	4.7	13	11.4	2.2	19
4	29.7	7.5	17	11.7	2.8	12
5	29.7	4.1	6	11.4	1.6	16
6	36.1	5.8	23	13.6	2.1	16
7	30.1	4.9	17	12.8	2.7	19
8	33.5	4.4	16	11.9	1.5	18
9	32.2	5.3	23	13.7	2.1	21
10[a]	33.3	---	1	15.5	2.0	3
	Grand mean (m/s)	S.E.M. (m/s)	N	Grand mean (m/s)	S.E.M. (m/s)	N
	32.2	1.0	9	12.6	0.3	9

[a] Values not used in calculation of grand mean and S.E.M. (Drewes, Landa, and McFall, 1978)

always consisting of two similar monophasic spikes separated by an interval of 1.2-2.0 ms (Fig. 2). These spikes were followed by a slower potential of variable waveform.

To demonstrate which, if any, of these potentials originated in the MGF we compared electrical recordings from undissected and dissected portions of the same animals. Responses to tactile stimulation of the head were identical whether recorded from the undissected posterior end of the worm (Fig. 3A; lower trace) or from the exposed ventral cord. If the segmental nerves were then cut close to their central connections with cord, both the second spike and slow potential were no longer recorded from the ventral nerve cord, but were still seen in recordings from the undissected posterior end (Fig. 3A). We infer from these results that only the first spike occurs in the ventral nerve cord and, given its rate and direction of conduction, that it arises from the MGF. The second spike appears to occur in a peripheral nerve and may represent activity in a large motor axon. Using dissected earthworms, Günther (1972) demonstrated 1:1 coupling of MGF spikes with spikes in identifiable giant motor axons in the segmental nerves. These motor axon spikes when recorded extracellularly from a peripheral nerve had a large amplitude and followed the MGF spike by approximately 1.5 ms. This value is within the range of interspike intervals in our recordings. The slow potential which follows the presumed motor axon spike (Figs. 2, 3A) may be that of a muscle in the body wall.

Fig. 2. Responses of the medial giant fiber (MGF) to tactile stimulation of the first few anterior segments. In each record the recording site for the upper trace was located 20 mm anterior to the site for the lower trace. (A) A light tactile stimulus evoked a response consisting of two spike potentials and a slower multiphasic potential. The initial MGF spike (first dot in each trace) was followed approximately 1.8 ms later by a second spike (second dot in each trace), probably from giant motor axons (Günther, 1972). The larger, slower potential was probably of muscular origin. (B) With strong tactile stimulation two MGF spikes were evoked (first and third dots). Each MGF spike was followed by a motor axon spike (second dot). The slow potentials, presumably of muscular origin, showed marked facilitation in B. This response was accompanied by a rapid longitudinal contraction of the anterior end of the animal. Time scale: 2 ms. (Drewes, Landa, and McFall, 1978)

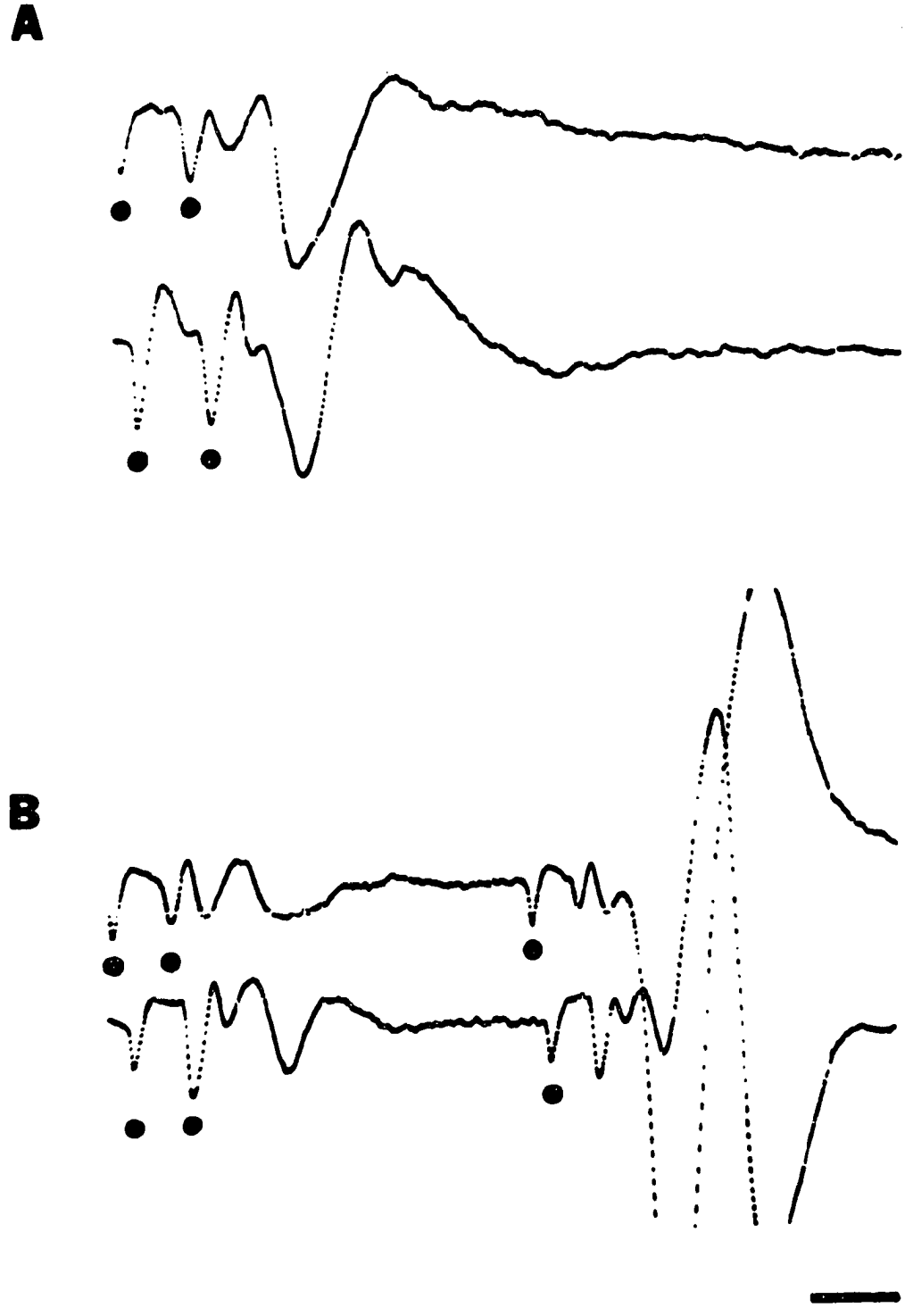
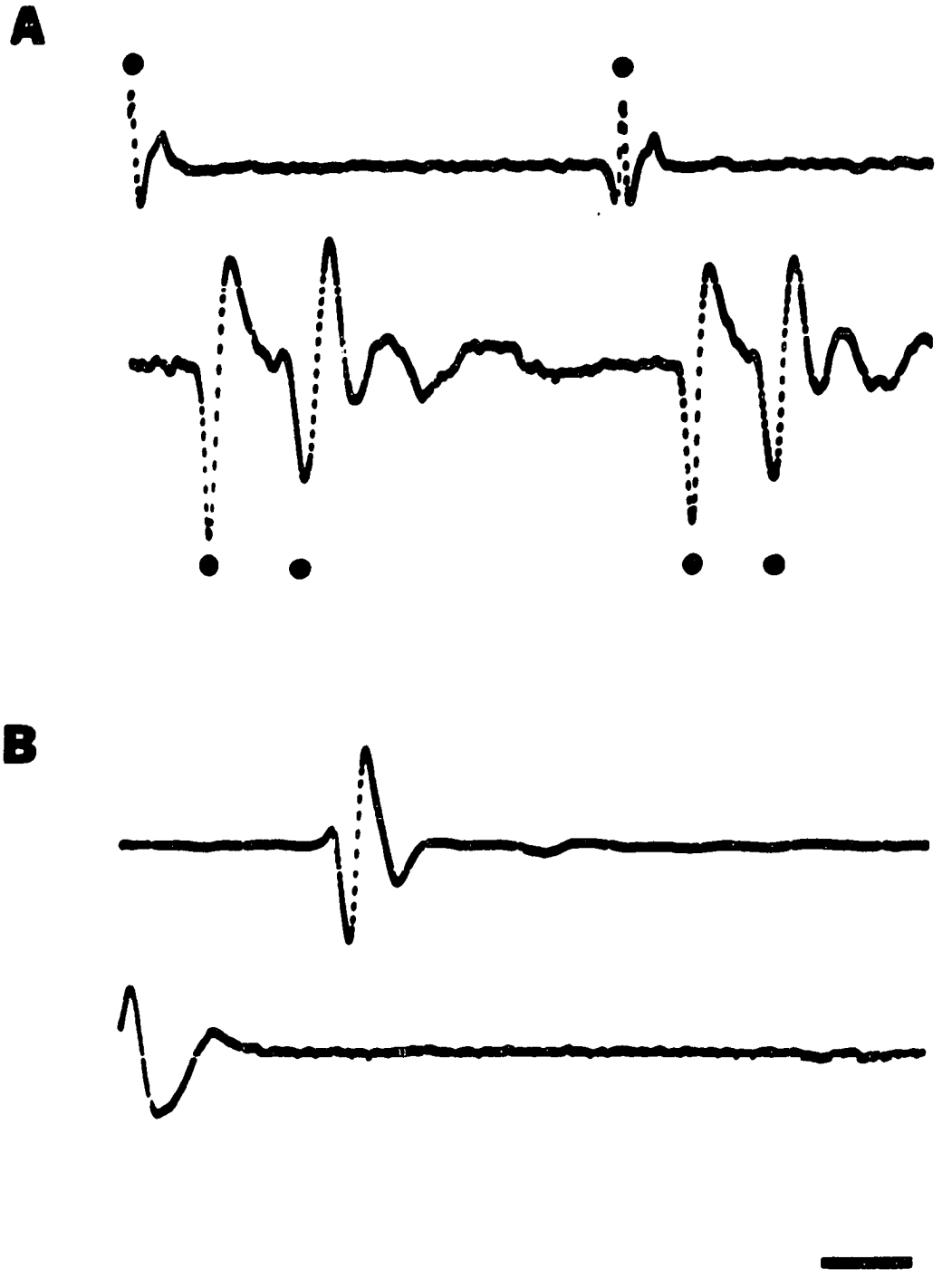

A
B

Fig. 3. Recording of giant fiber activity in partially dissected, restrained earthworms in response to tactile stimulation. (A) Strong tactile stimulation of the anterior end of the animal evoked a series of two MGF spikes. In the upper trace a pair of electrodes was in contact with the ventral nerve cord in the centrally dissected portion of the animal (all segmental nerves were severed in this region). The two spikes (dots) arise centrally from the medial giant fiber. In the lower trace a pair of electrodes simultaneously recorded activity (50 mm away) in the intact, posterior end of the animal. The two MGF spikes (first and third dots) were followed by spikes (second and fourth dots) presumably occurring in a peripheral motor axon. The smaller, slower potentials may be of muscular origin. (B) A strong tactile stimulus to the posterior end of the animal evoked a single spike, arising centrally from the lateral giant fiber. In the lower trace a pair of recording electrodes was in contact with the intact posterior end of the animal. In the upper record another pair of electrodes (50 mm away), made direct contact with the exposed ventral nerve cord in the central portion of the animal. Time scale: 2 ms. (Drewes, Landa, and McFall, 1978)


A
B

The number of MGF spikes evoked in intact animals in response to a tactile stimulus was roughly proportional to the strength of stimulation. Light tactile stimulation evoked a single MGF spike along with the presumed motor axon spike and muscle potential (Fig. 2A). In 29 tests single MGF spikes were never accompanied by observable longitudinal contraction of the animal. Stronger tactile stimulation evoked a series of two or more MGF spikes (Fig. 2B). Each series was accompanied by facilitation of the slower potentials and marked longitudinal contraction.

An interesting characteristic of MGF activity was that the second and subsequent spikes in a train were always propagated at a faster rate than the initial spike. Bullock (1951) observed similar increases in conduction rate in isolated nerve cords. He found that this 'facilitation of conduction' was maximal 6 ms after a previous spike, but was still perceptible after 100-200 ms. In our study paired MGF spikes were recorded from eight animals. The mean rate for the second MGF spike was 39.0 ms ± 2.1 S.E.M. (preceding interspike interval $\leq$ 15 ms). Using a paired-difference t-test this value was significantly greater ($P < 0.001$) than the mean rate of the first spike (mean = 31.9 m/s ± 1.7 S.E.M.).

Lateral Giant Fiber Activity

Activity evoked by tactile stimulation of the terminal few segments of intact animals was always conducted in a posterior-anterior direction at a rate matching that of the lateral giant

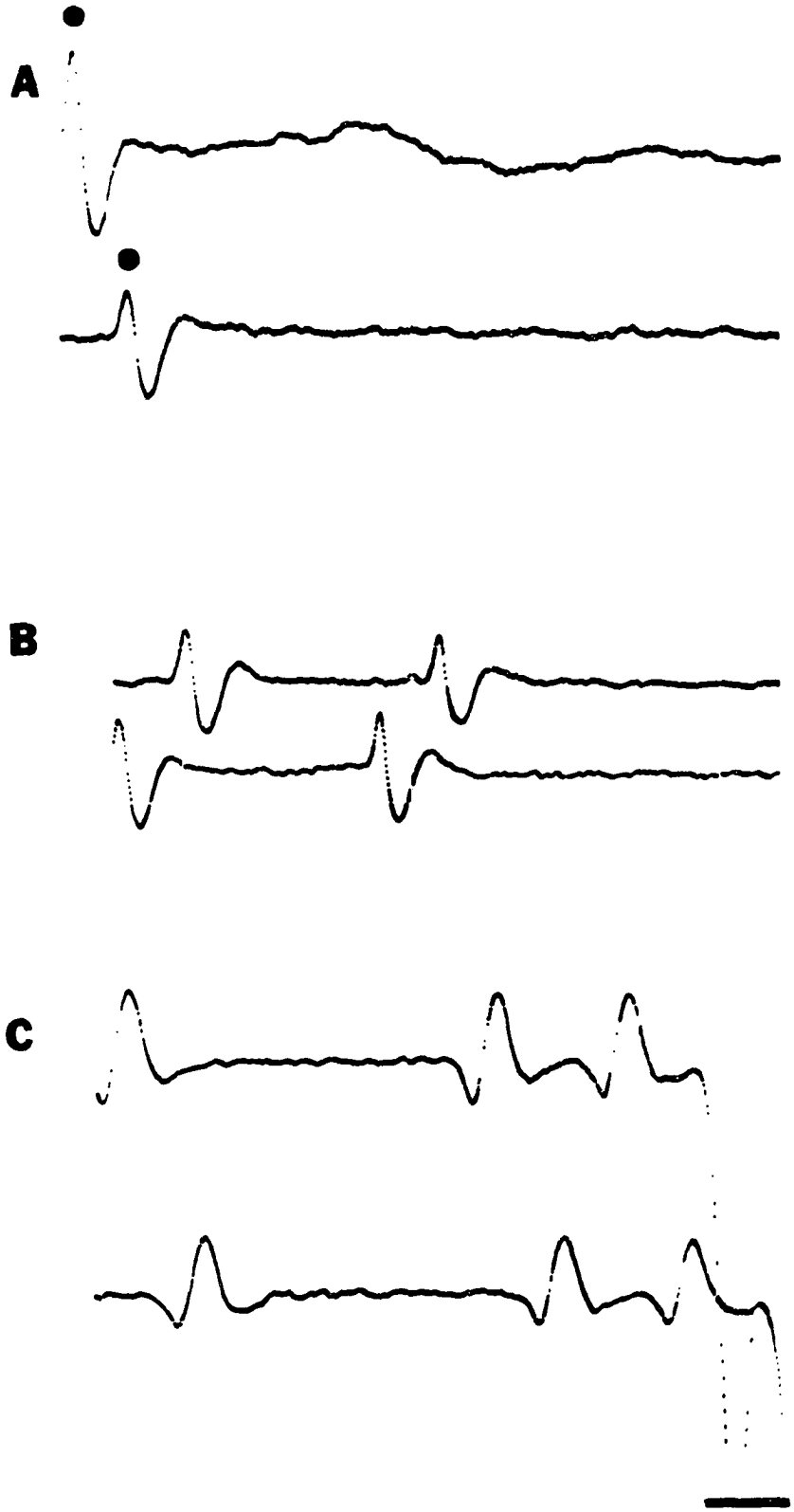
fibers (LGF). The activity invariably consisted of simple triphasic spikes (Fig. 4).

To demonstrate that these spikes arose from the lateral giant fiber we compared recordings from dissected and undissected portions of the same animal using procedures described in the previous section. Spikes recorded from the exposed ventral nerve cord in the central portion of the animal were identical to those recorded in the undissected posterior end (Fig. 3B). Cutting the segmental nerves had no effect on the waveform of the spikes. Therefore we conclude that the spikes occur in the ventral nerve cord and, considering conduction parameters, that they arise from the LGF.

The number of LGF spikes evoked in intact animals by tactile stimulation was roughly proportional to the stimulus strength. With strong stimulation high-frequency trains of LGF spikes were evoked (spike frequencies up to 500/s). Single spikes, evoked in 33 tests of eight animals (Fig. 4A), were never accompanied by longitudinal contraction of the animal. In 12 tests paired spikes were evoked (Fig. 4B). In six of these tests slight longitudinal contraction was observed; in the other six no contractions were observed. Trains of three or more spikes (Fig. 4C) were invariably accompanied by large and slower potentials (presumably of muscular origin) and by rapid longitudinal contraction of the animal.

As with the MGF spikes, the second and subsequent LGF spikes

Fig. 4. Responses of the lateral giant fiber (LGF) to tactile stimulation of the tail. (A) A light tactile stimulus evoked a single spike (dot) which is recorded at two different sites in the posterior half of the animal. The recording site for the upper trace was 20 mm more posterior than that for the lower trace. (B) A tactile stimulus evoked a pair of LGF spikes, the interspike interval being approximately 7 ms. The recording site for the upper trace was located 20 mm anterior to the site for the lower record. (C) A strong stimulus evoked three LGF spikes. The recording site for the upper trace was 20 mm posterior to the site for the lower trace. In each trace the third spike was followed by a very large downward deflection (not shown), presumably a muscle potential from the body wall. The animal responded with a rapid longitudinal contraction. Time scale: A-C = 2 ms. (Drewes, Landa, and McFall, 1978)


A
B
C

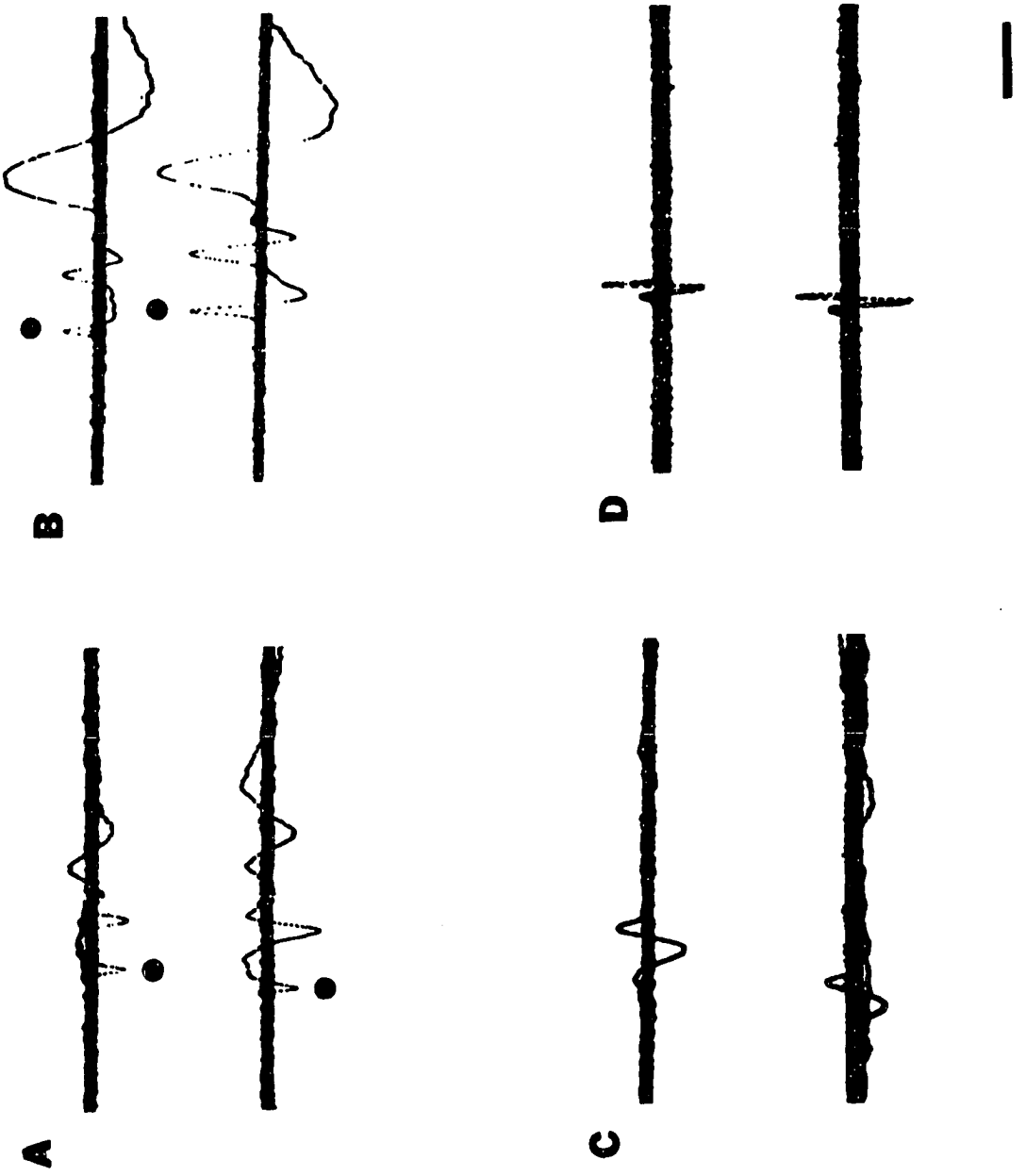
in a train were propagated at a faster rate than the initial spike. In nine animals the mean rate for the second spike was 14.0 m/s ± 0.4 S.E.M. (preceding interspike intervals $\leq$ 15 ms). Using a paired difference t-test this value was significantly greater ($P < 0.001$) than the rate of the initial spike (mean = 11.9 m/s ± 0.4 S.E.M.).

Giant Fiber Activity in the Absence of Experimenter-Applied Stimuli

Previous approaches have not allowed investigators to determine if giant fiber spikes occur in earthworms in the absence of experimenter-applied stimulation. The possibility that such spikes may occur during locomotory movements was tested in the freely moving animals. Approximately 15-20 min of recordings were obtained for each animal, during which both medial and lateral giant fiber spikes were seen.

A total of 49 MGF spikes were recorded (Fig. 5A, B). These usually occurred during forward peristalsis or local movements of the head, though most locomotory movements were not accompanied by MGF activity. Conduction was in an anterior-posterior direction at a grand mean rate of 30.5 m/s (± 1.4 S.E.M.). We observed 45 instances of single MGF spikes and two instances of paired spikes (interspike intervals less than 20 ms). Each MGF spike was followed by a presumed motor axon spike and muscle potential.

Fig. 5. Giant fiber activity in the absence of any experimenter-applied stimulation. (A, B) MGF spiking activity (dot) was recorded at two different sites (20 mm apart) during locomotory movements. The lower trace in (A) and the upper trace in (B) are from anterior recording sites. In both records the activity, consisting of a single MGF spike, was conducted in an anterior-posterior direction. (C, D) LGF activity was recorded at two sites (upper traces, anterior; lower traces, posterior). Time scale: A-C = 2 ms; D = 5 ms. (Drewes, Landa, and McFall, 1978)


B
D
A
C

A total of 22 LGF spikes were also recorded in the absence of experimenter-applied stimulation (Fig. 5C, D). Conduction was in a posterior-anterior direction at a grand mean rate of 12.7 m/s (± 1.4 S.E.M.). These spikes, though infrequent, usually occurred during reverse peristalsis and rarely during forward peristalsis. We observed 18 instances of single LGF spikes and two instances of paired spikes.

DISCUSSION

Prior to undertaking any detailed investigation of how giant fiber activity is expressed in behavior, it was necessary to characterize, for intact animals, giant fiber parameters previously described only in isolated or semi-intact preparations. We have confirmed that the difference in the direction of spike conduction, characteristic of the medial and lateral giant fibers in dissected preparations (Stough, 1930; Rushton, 1946; Bullock, 1945), also occurs in the intact animal. We have also confirmed that there are increases of up to 20% in conduction rate of closely spaced giant fiber spikes (Bullock, 1951).

Several parameters of giant fiber spikes in intact animals differ from those in dissected preparations. The conduction rates of giant fibers in intact animals (MGF, 32.2 m/s; LGF, 12.6 m/s), although within the very broad range given by Bullock (1945), are considerably greater than values given in detailed studies of conduction rates in isolated cords. Lagerspetz and Talo (1967), who have done an extensive study of the effect of temperature on giant fiber conduction rates, give means of about 16 and 9 m/s for the medial and lateral giant fibers, respectively, in isolated cords (22.4 °C). They also tabulated values obtained by other workers (range 11.2-27.8 m/s for MGF; 5.5-12 m/s for LGF). In work preparatory to this study we have obtained values

similar to Lagerspetz and Talo in isolated cords from our animals. These are relatively low rates, compared to values obtained from intact animals, and may be attributed to the trauma (e.g. disruption of cirulatory system or change in chemical environment) associated with dissection. Similarly, the capability of intact animals to discharge giant fiber spikes at high frequencies (up to 500/s) in response to tactile stimulation exceeds that seen in isolated cords using electrical stimulation (absolute refractory period = 2-4 ms; Bullock, 1945).

The relationship of giant fiber activity to longitudinal contraction in intact animals appears similar to that seen in dissected preparations (Roberts, 1962a; 1966). Roberts's studies showed that a single giant fiber spike can evoke a very small longitudinal twitch and that large contractions are seen only in response to repetitive giant fiber discharge. Therefore, Roberts suggested that the escape response of the earthworm may be graded, its magnitude depending on the number of giant fiber spikes which occur. Our studies of intact animals support this idea. Single spikes were never accompanied by observable longitudinal contraction. Paired spikes, on the other hand, were sometimes adequate in evoking observable contractions, and three or more spikes consistently evoked such contractions.

The occurrence of single and paired giant fiber spikes in freely moving animals in the absence of experimenter-applied

stimulation suggests that either sensory inputs associated with normal locomotion over an irregular substrate are sufficient to excite giant fibers or giant fiber activity may occur as part of an internally generated motor program. The infrequency of giant fiber spikes relative to locomotor cycles argues against the latter; current data, however, are insufficient for a final determination.

This study represents the first phase of a study of neural correlates of behavior in intact, freely moving earthworms. Further development of the apparatus will provide recording of behavioral and electrophysiological data under the control of a microcomputer. Since the recording technique allows monitoring of electrophysiological events and behavior for relatively long periods, it may be particularly useful in studying developmental or possible daily/seasonal variations.

SUMMARY

1. An approach is described for recording and characterizing giant nerve fiber activity in intact, freely moving earthworms.

2. Medial giant fiber (MGF) spikes were conducted in an anterior-posterior direction at a mean rate of 32.2 m/s; lateral giant fiber (LGF) spikes were conducted in a posterior-anterior direction at a mean rate of 12.6 m/s.

3. Rates of giant fiber spike conduction and maximal frequencies of firing (up to 500/s) in intact animals were higher than values previously reported in isolated preparations.

4. MGF spikes were followed 1:1 by presumed giant motor axon spikes and facilitating muscle potentials.

5. Single MGF or LGF spikes evoked by applying tactile stimulation were not accompanied by longitudinal contraction, but a series of two or more MGF spikes or three or more LGF spikes were accompanied by such contractions.

6. MGF and LGF spikes occurred infrequently during locomotory movements in the absence of any experimenter-applied stimulation, suggesting that sensory inputs associated with normal locomotion over an irregular substrate are sufficient to excite giant fibers.

PART II. LONGITUDINAL VARIATIONS IN THE PROPERTIES OF THE MGF-GMNI PATHWAY

INTRODUCTION

The nervous systems of a wide variety of invertebrate groups possess unusually large diameter ("giant") nerve fibers which, when activated, mediate rapid escape behavior. The presence of giant nerve fibers in earthworms and their involvement in the rapid longitudinal withdrawal ("startle") response of the animal has been established and confirmed by many investigators (for reviews see Bullock and Horridge, 1965; Gardner, 1976). In the earthworm, Lumbricus terrestris, two functionally different giant fiber systems, the medial (MGF) and lateral (LGF) giant fiber systems, are known (Bovard, 1918; Stough, 1930; Bullock, 1945). The MGF system, the subject of this study, consists of a single through-conducting interneuronal pathway, formed by the tandem arrangement of large segmental interneurons (Mulloney, 1970; Günther, 1975).

The sensory field for activation of the MGF includes approximately the anterior one-third of the animal (Günther, 1973). Based on visual observations, the behavioral response to touch within this sensory field consists of a longitudinal shortening of the animal (Bovard, 1918; Rushton, 1946). By monitoring the mechanical tension accompanying this shortening, Roberts (1962a) showed that the response was graded, depending on the number of

giant fiber spikes. Additionally, other investigators (Bovard, 1918; Rushton, 1945) have mentioned that escape contractions are often accompanied by flattening of the tail, and have suggested that the flattening may be mediated by the MGF system. Until the studies of GÜnther (GÜnther and Walther, 1971; GÜnther, 1971, 1972) however, very little was known about the structure and function of the specific efferent neuronal elements which produce these behavioral responses.

GÜnther and Walther (1971) described 3 pairs of giant motor neurons (designated GMN1) which are coupled to the MGF in each segment, one pair of GMN1's for each pair of segmental nerves. Each GMN1 exits the ventral nerve cord via its contralateral segmental nerve. Due to the large diameter of GMN1 axons, large amplitude extracellular recordings of GMN1 spikes are obtainable from segmental nerves of dissected preparations. GÜnther (1972) showed that each MGF spike is coupled, after a brief latency, to a GMN1 spike. His electrophysiological recordings from the body wall indicated that at least some of the GMN1's innervate the longitudinal muscle layer, providing a basis for the rapid longitudinal contractions observed during escape. Using noninvasive techniques, we recently demonstrated that MGF and GMN1 spikes, as well as GMN1-mediated muscle potentials, can be simultaneously monitored at multiple sites on the ventral surface of freely moving earthworms (Drewes, Landa, and McFall, 1978). This

provides the possibility of direct and detailed analysis of the central neural and efferent correlates of escape behavior at different sites along intact animals. In this study we have focused on regional variations in MGF-mediated escape responses.

MATERIALS AND METHODS

Electrophysiology

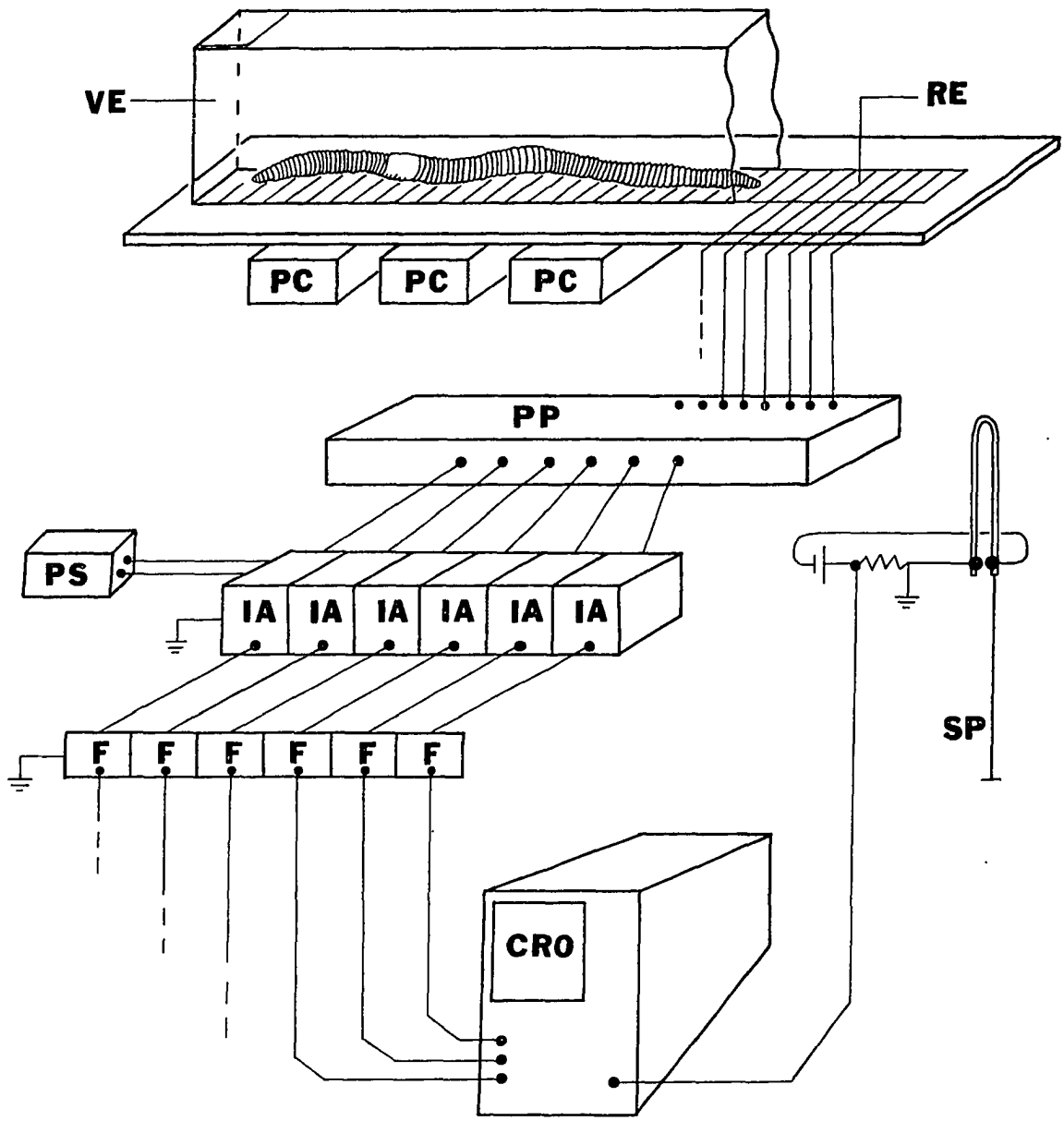
Mature earthworms, obtained locally, were maintained in the dark at 15 °C in Magic Worm Bedding (Magic Worm Bedding Company, Inc., Amherst Junction, Wisconsin). Before testing, worms were rinsed in deionized water to remove particles of bedding and were transferred to the experimental chamber consisting of a linear array of 100 Ag-AgCl wire electrodes (26 gauge), spaced 2 mm apart and mounted on a plexiglass frame (Fig. 6). Animals were allowed to move freely within a vinyl enclosure and allowed to acclimate for approximately 15-20 min before testing. The animals were moistened periodically with drops of deionized water. All experiments were conducted at room temperature (22-23 °C).

Recording sites along an animal were selected according to its position on the electrode array. Signals from any six electrode pairs were routed via a patch panel system to instrumentation amplifiers (Fig. 6). Signals from the single-ended outputs of the instrumentation amplifiers were filtered and displayed on a storage oscilloscope. Tactile stimulation was applied to the animal with a hand-held probe which was also used for triggering single sweeps of the oscilloscope. The delay from mechanical contact of the probe tip to triggering of the oscilloscope was determined in independent tests and ranged from 5-40 ms (mean 20 msec).

Longitudinal contractions associated with earthworm escape

Fig. 6. Diagram of stimulating and recording apparatus. The earthworm crawls within a rectangular clear vinyl enclosure (VE) (height, 5.0 cm; length, 20.0 cm; width, 1.4 cm). Signals picked up by recording electrodes (RE) from multiple sites along the ventral surface of the animal are routed via the patch panel (PP) to six instrumentation amplifiers (IA) (AD521J, Analog Devices, Inc., Norwood Mass.) requiring ± 15V power supplies (PS). High (3 KHz) and low (100 Hz) frequency filters (F) were built into the coaxial cables connecting the instrumentation amplifiers to a Tektronix 5111 storage oscilloscope (CRO) equipped with multichannel amplifiers.

Tactile stimulation of the animal with the tip of the stimulus probe (SP) (tip diameter = 0.4 mm) closed contacts which were in series with a simple battery circuit, thus triggering a single sweep on the oscilloscope. The tip of the probe was insulated from the battery circuit to prevent possible electrical stimulation of the body wall. Photocells (PC) were used to measure behavioral responses (see Fig. 7).

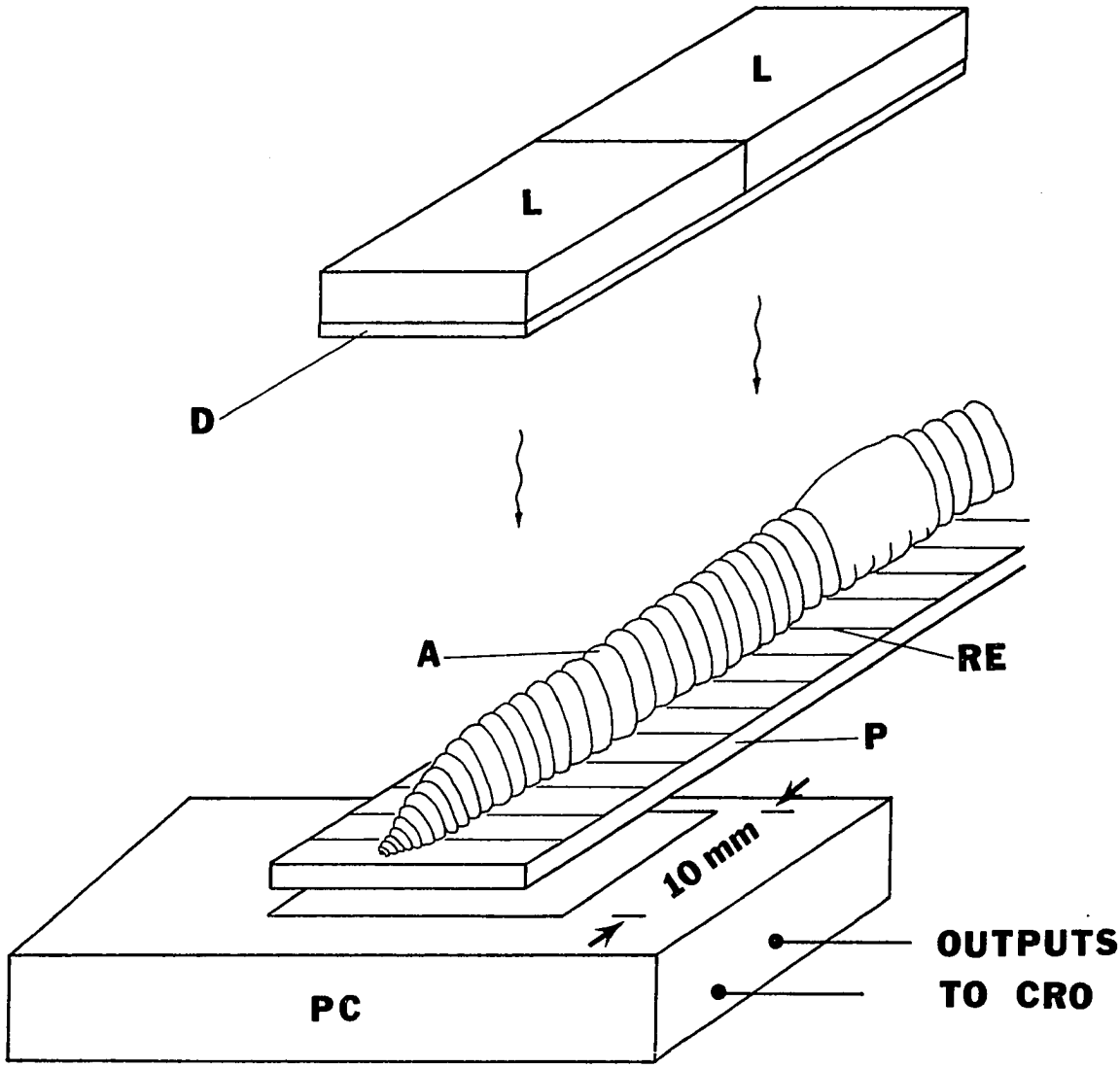

VE
RE
PC
PC
PC
PP
PS
IA
IA
IA
IA
IA
IA
F
F
F
F
F
F
SP
CRO

behavior produce corresponding changes in animal width. We examined regional variations in animal width during escape by using three independently movable photocells, each connected to a different channel of the oscilloscope. The light-sensitive region of each photocell was restricted to an area 1.0 cm long and 1.4 cm wide (same width as enclosure). When located under an animal on the array, each photocell provided a monitor of width for approximately ten segments of the animal's body (Fig. 7). Increases in animal width produced changes in the D.C. outputs from the photocells which were displayed as upward deflections on an oscilloscope. The entire system was calibrated by adjusting the sensitivity of the oscilloscope amplifiers using cylindrical objects of known diameter. The responsiveness of the calibrated system was essentially linear over the experimental range. Response time of the photocells was essentially instantaneous. Approximately 15 min were allowed between behavioral tests.

Anatomy

Numerous studies of the MGF in several species of earthworms indicated that giant fiber diameter is not uniform over its length (for review see Bullock and Horridge, 1965). We examined freshly dissected living nerve cords from Lumbricus terrestris and related variations in giant fiber diameter to variations in conduction velocity recorded from intact animals. Nerve cords were pinned ventral side down in a transparent dish filled with saline (Drewes

Fig. 7. Diagram of the arrangement for monitoring behavioral responses of the earthworm. The animal (A) laying across recording electrodes (RE) screens a portion of the 10 mm long light-sensitive region of the photocell (PC). The photocells (G.E. model 8PV1) were scavanged from obsolete colorimeters. Illumination of the entire recording chamber was provided by two 6 Volt D.C. lanterns (L) (bulb type PR-13) and a diffusor (D) placed 0.6 m above the animal.


L
L
D
A
RE
P
10 mm
OUTPUTS
TO CRO
PC

and Pax, 1974a). Selected segments were stretched to a length of 1.0 mm. Preparations were viewed and photographed through a dissecting microscope using substage illumination. Measurements were obtained from equal photographic enlargements of the nerve cords and a stage micrometer. Due to the cylindrical shape of the translucent giant fibers and the surrounding myelin-like sheath, measurement accuracy was limited to ± 5 μ.

RESULTS

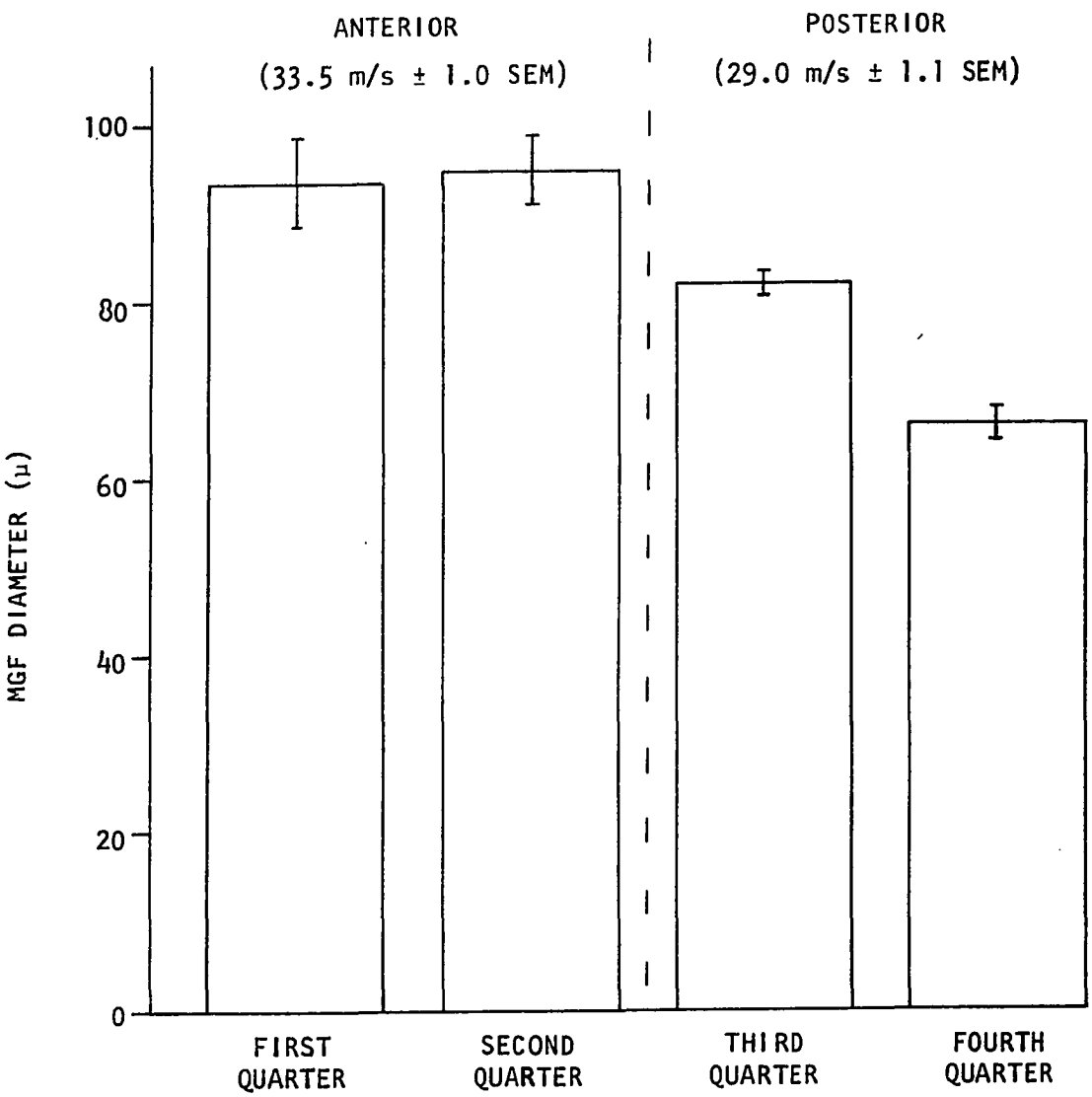
Regional Variations in MGF Conduction Rate and Diameter

Results summarizing observed regional variations in diameter and conduction rate are given in Fig. 8. MGF diameter is greatest (approximately 95 μ) in the anterior 2 quarters of the animal, tapering in the posterior to approximately 70% of maximum. These differences were related to regional differences in MGF conduction rate.

Single MGF spikes were evoked by light tactile stimulation of the head, and conduction velocities were determined by measuring peak-to-peak time lag between spikes recorded from selected sites separated by known distances. The grand mean for MGF conduction rates measured in the anterior one-half of eight animals was 33.5 m/s; the grand mean conduction rate in the posterior one-half was 29.0 m/s. Using a paired difference t-test (Mendenhall, 1968), the mean conduction rates in the anterior and posterior halves of the animals were found to be significantly different ($p < 0.001$).

We also tested for regional variations in the ability of the MGF to facilitate its conduction rate, a property previously described for giant fibers in isolated nerve cords (Bullock, 1951) and in intact animals (Drewes, Landa, and McFall, 1978). Tactile stimulation of sufficient strength to evoke pairs of MGF spikes was applied and conduction rates of second spikes compared

Fig. 8. Regional variations in MGF diameter and conduction rates. Bars indicate mean diameters (vertical lines represent S.E.M.; n = 5 animals) in each quarter (approximately 40 segments) of the animal. The grand mean conduction rates (n = 8 animals) are shown for anterior and posterior halves.


ANTERIOR
(33.5 m/s ± 1.0 SEM)
POSTERIOR
(29.0 m/s ± 1.1 SEM)
MGF DIAMETER (μ)
100
80
60
40
20
0
FIRST QUARTER
SECOND QUARTER
THIRD QUARTER
FOURTH QUARTER

to those of first spikes. Data were taken only from MGF spike pairs with interspike intervals ≤ 15 ms. The grand mean conduction rate for second spikes in the anterior one-half of the animal was 39.6 m/s ± 2.6 S.E.M. (n = 4), representing approximately an 18% increase over the conduction rates for first spikes. In the posterior one-half of the animal the grand mean conduction rate for second spikes was 33.6 m/s ± 1.1 S.E.M. (n = 4), representing a 16% increase over the conduction rate for first spikes. These results suggest that the capability of the MGF to facilitate its conduction rate is similar over its length.

Regional Variations in MGF-GMN1 Coupling

Previous electrophysiological studies of giant fiber activity from dissected animals (Günther, 1972) and from the ventral surface of intact earthworms (Drewes, Landa, and McFall, 1978) indicated that each MGF spike is followed after approximately 1-2 ms by a GMN1 spike. In the present study simultaneous recordings taken from intact animals indicated that MGF-GMN1 coupling is always 1:1 in all regions of the body and that the GMN1 spike is probably a compound spike derived from synchronously occurring action potentials in left and right GMN1 axons from several neighboring segmental nerves. This interpretation is supported by the fact that GMN1 spike waveforms occasionally appear as complex spike potentials rather than simple monophasic or diphasic spikes (e.g., upper 2 traces in Fig. 10). Such waveforms would be expected if

GMN1 spikes in neighboring segmental nerves occurred slightly out of phase with one another.

With regard to the temporal aspects of MGF-GMN1 coupling, no significant variations were seen along the animal. For single spike responses, time lags from the peak of the MGF spike to the largest peak of the GMN1 spike were always within the range of 1.0 - 2.0 ms at all locations. A slight decrease (approximately 0.1 - 0.2 ms) in MGF-GMN1 lag time was seen for the second of two closely spaced MGF responses. These decreases were similar in all regions of intact animals and were comparable to those reported by GÜnther (1972) in studies of isolated nerve cords.

Regional Variations in GMN1-Mediated Muscle Potentials

As shown in Figs. 9-11, each GMN1 spike, after a 1-2 ms delay, gives rise to discrete muscle potentials probably occurring in the longitudinal muscle layer (GÜnther, 1972). Although somewhat variable in amplitude in all regions, muscle potentials evoked by a single GMN1 spike tended to be of greatest amplitude in the anterior 50-60 segments and terminal 15-20 segments of the animal (Fig. 9).

With repeated firing, GMN1-mediated muscle potentials can show marked facilitation of amplitude (Fig. 11). Our results indicate that the degree of facilitation is not the same for different regions of the animal, facilitation being most pronounced

Fig. 9. Simultaneous recordings of MGF spikes at six sites along the animal in response to a very light touch to the head. The recording site for the upper trace is approximately segment 140 and for the lower trace is approximately segment 25. At each recording site a single MGF spike is followed after approximately 2 ms by a GMN1 spike (dots). GMN1 spikes are followed by slower muscle potentials which are usually of greatest amplitude in the 50 most anterior and 20 most posterior segments of the animal.

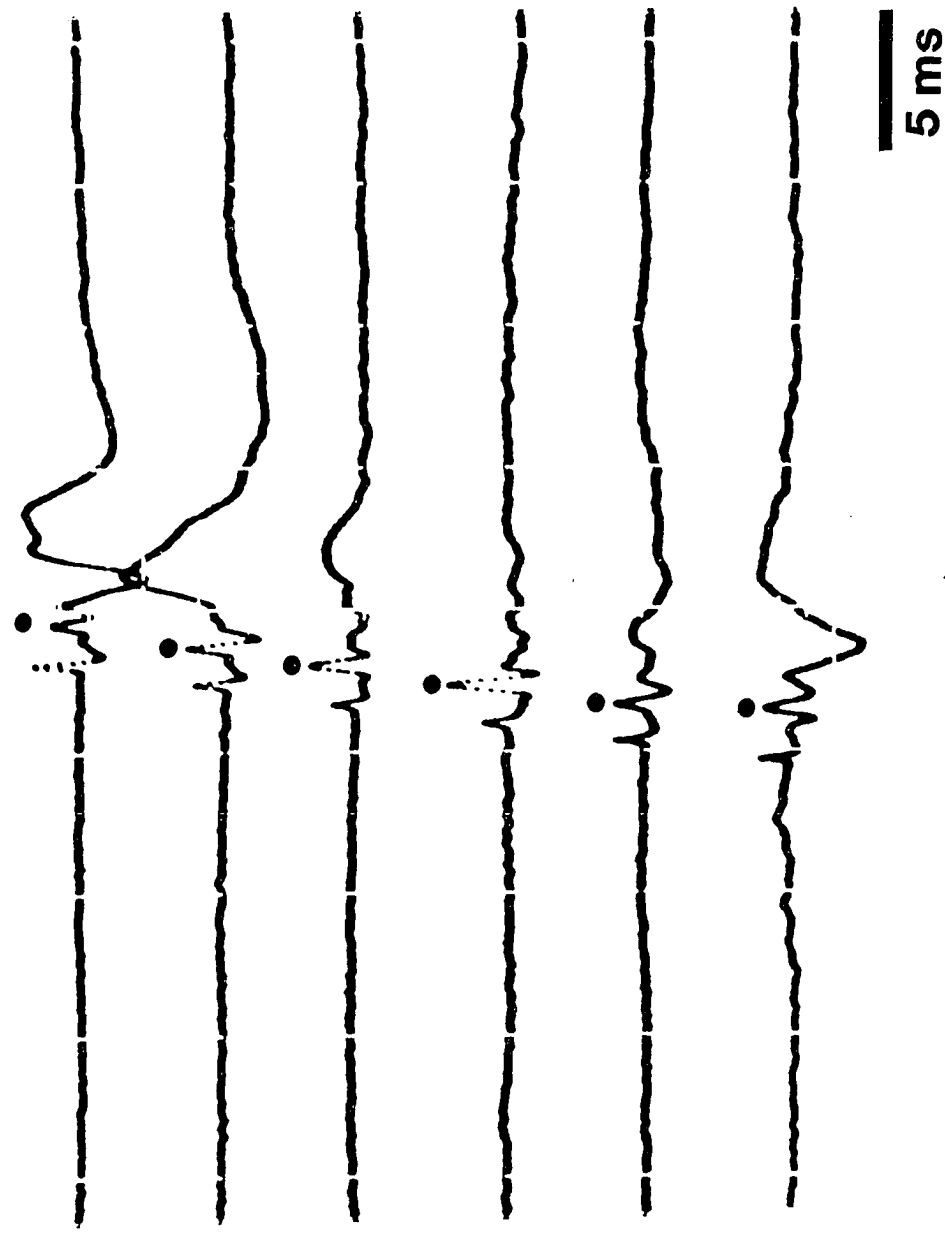

5 ms

Fig. 10. Responses to widely spaced MGF spikes evoked by a light touch to the head. Recording sites are 20 mm apart, the most anterior (lower trace) being at approximately segment 30 and the most posterior (upper trace) at approximately segment 140. A pair of MGF spikes with an interspike interval of about 19 ms are shown. Note that no facilitation of GMN1-mediated muscle potentials occurs. In fact, in the most posterior record (upper) a slight antifacilitation is seen.

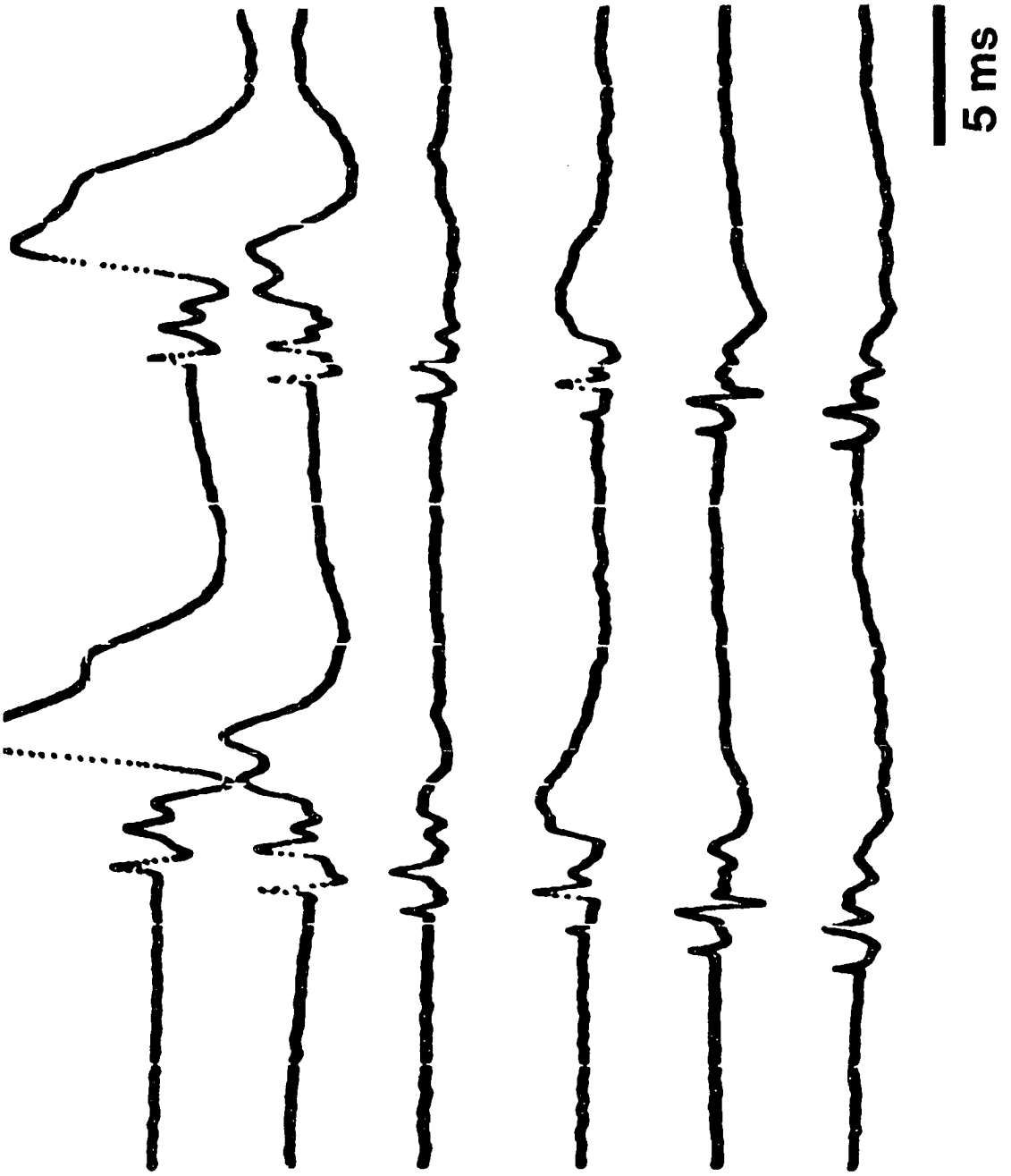
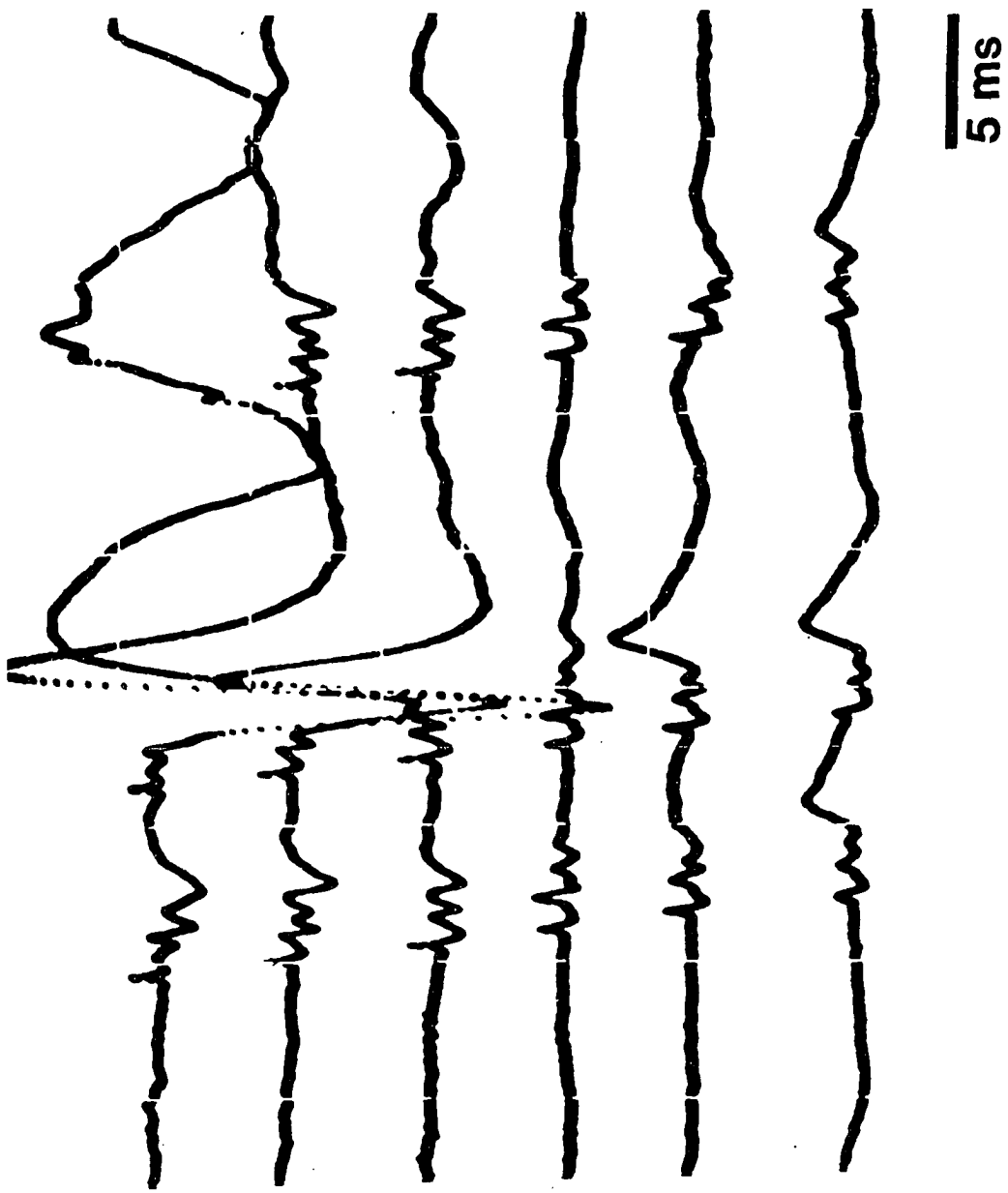

5 ms

Fig. 11. Responses to closely spaced MGF spikes evoked by a moderate touch to the head. Recording sites are 20 mm apart, the most anterior (upper trace) being at approximately segment 25 and the most posterior (lower trace) at approximately segment 140. Three MGF spikes are shown. The interspike interval for the first two MGF spikes is about 7 ms. Note that the second GMN1-mediated muscle potential facilitates most in the three anterior recordings and less in the posterior recordings. The third set of MGF and GMN1 spikes, preceded by an interspike interval of approximately 15 ms, is accompanied by smaller muscle potentials.


5 ms

anteriorly and diminishing sharply in posterior regions. Figure 12 shows the quantitative relationship between amount of facilitation of muscle potentials and location along the animal. Since the amplitude of second GMN1-mediated muscle potentials was often one to two orders of magnitude greater than the amplitude of the first, we found it convenient to express the amount of facilitation as the log of the ratio of the second muscle potential amplitude to that of the first. Data were taken only from tests in which GMN1 interspike intervals were between 4-8 ms. In the anterior 30 segments the amplitudes of second muscle potentials averaged nearly twenty times greater than those of first muscle potentials. In the posterior region (segments 121-150+) second muscle potentials averaged only about three times greater in amplitude than those of first muscle potentials.

While testing for regional variations in GMN1-mediated muscle activity, we also observed that the amount of facilitation of these muscle potentials was dependent on GMN1 interspike intervals (e.g., see Figs. 10 and 11). This relationship is quantified in Fig. 13 for the region of the animal between segments 31-60. As GMN1 interspike interval increased, the amount of facilitation decreased substantially. With relatively long interspike intervals (12-16 ms), only very slight facilitation was seen. A similar dependence of amplitude facilitation on GMN1 interspike interval was seen at all locations along the animal.

Fig. 12. Regional variations in the facilitation of GMN1-mediated muscle potentials. Data are from paired GMN1 responses in which GMN1 interspike intervals were relatively short (4-8 ms). The amount of facilitation of GMN1 muscle potentials is expressed as the log of the ratio of the second muscle potential amplitude to the first. Each bar indicates a grand mean of five animals; vertical lines indicate the range of individual means.

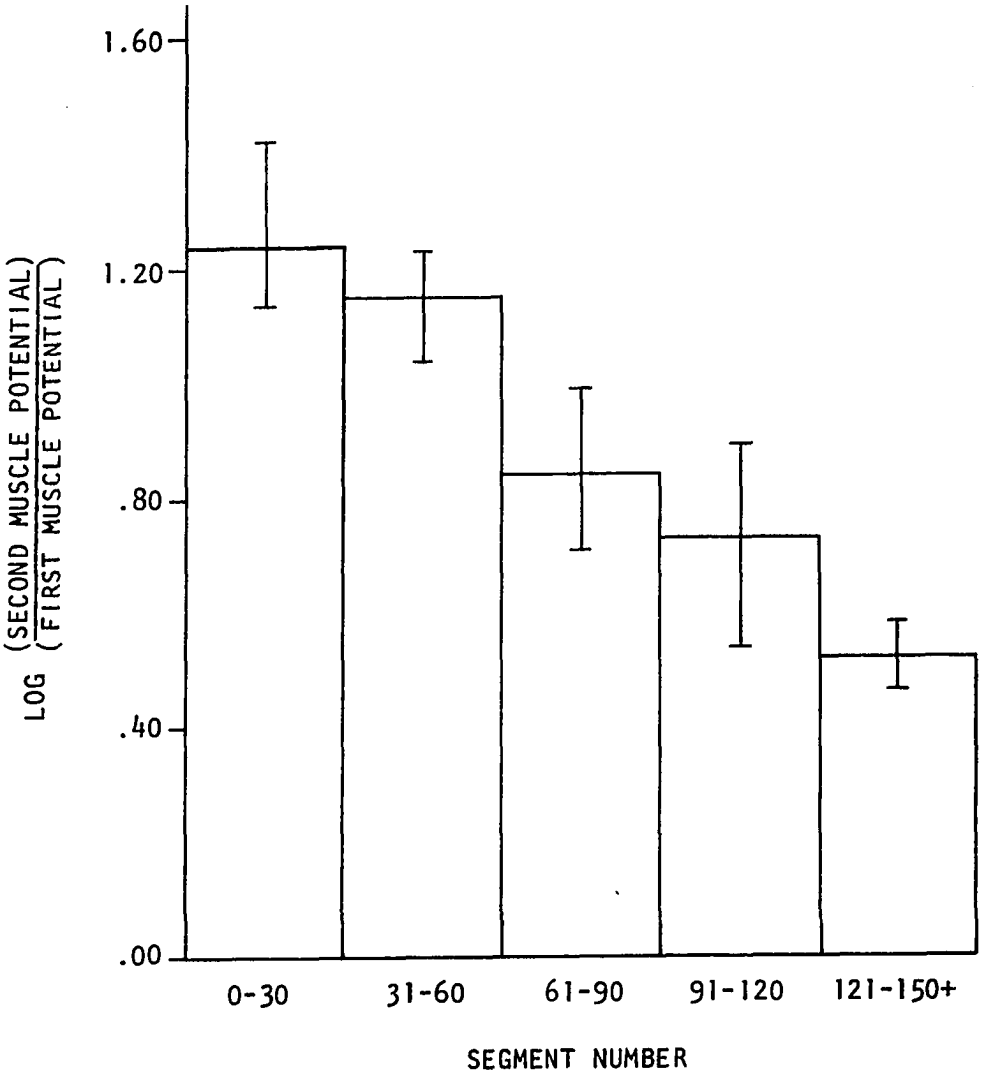
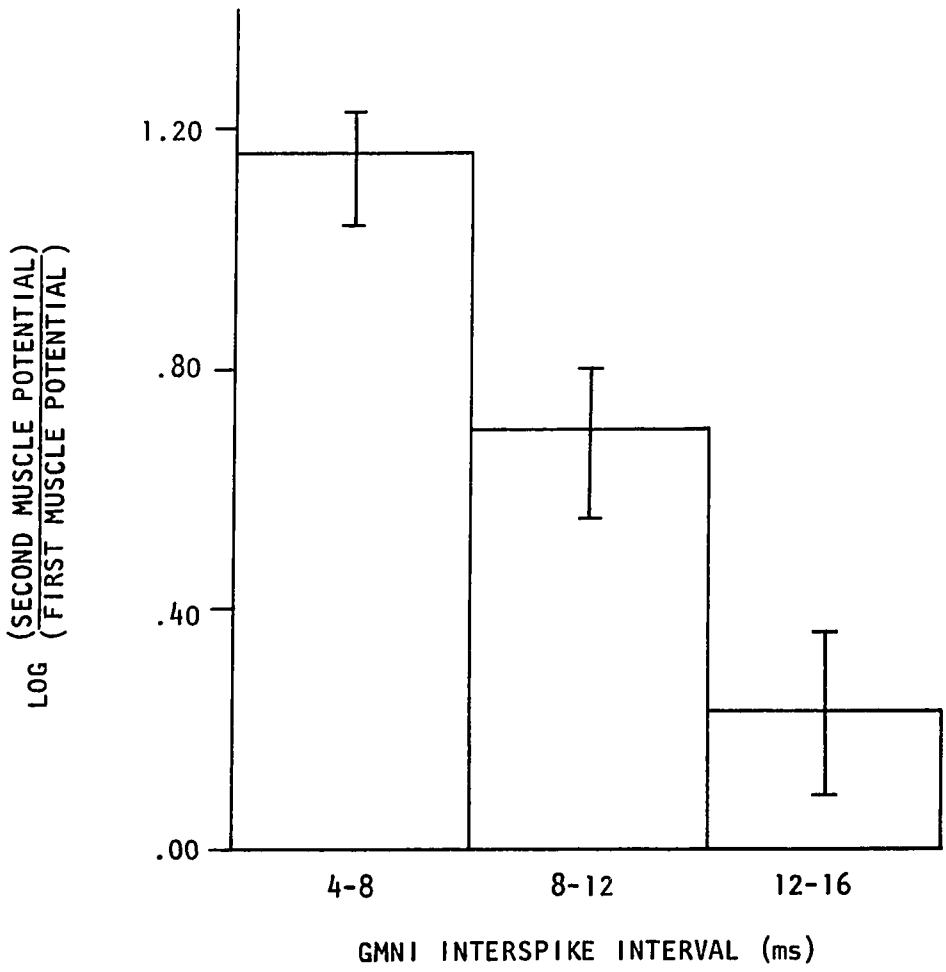

1.60
1.20
.80
.40
.00
LOG (SECOND MUSCLE POTENTIAL / FIRST MUSCLE POTENTIAL)
0-30
31-60
61-90
91-120
121-150+
SEGMENT NUMBER

Fig. 13. Relationship between the amount of muscle potential facilitation and GMN1 interspike interval. All data are from the region between segments 31 and 60. Each bar indicates a grand mean for six animals; vertical lines indicate the range of individual means from the animals.


LOG (SECOND MUSCLE POTENTIAL / FIRST MUSCLE POTENTIAL)
1.20
.80
.40
.00
4-8
8-12
12-16
GMNI INTERSPIKE INTERVAL (ms)

Regional Variations in MGF-Mediated Behavior

Although each MGF spike is accompanied by a GMN1 spike and a measurable muscle potential, a single MGF spike is not sufficient, at least under laboratory conditions, to evoke an observable longitudinal contraction. Instead, repeated spiking of the MGF is required for appreciable contraction to occur, the magnitude of response being related to the number and frequency of MGF spikes (Roberts, 1962a; Drewes, Landa, and McFall, 1978). Our results, based on data from five animals, indicate that there are regional differences in both quantity (magnitude) and quality of MGF-mediated behavioral responses. Except in the tail region, the most obvious MGF-mediated behavioral response to repeated firing of the MGF is a rapid longitudinal shortening of the animal.

During responses to a series of three or more MGF spikes, longitudinal shortening was most pronounced in the anterior one-third of the animal (Fig. 14). For any particular series of spikes the amount of shortening in the middle of the animal (e.g., segments 60-90) was always less (usually about one-half) than that occurring in the anterior (segments 10-40) (see Table 2). In tests during which only a few spikes were evoked, responses in the middle were generally undetectable. Often, in tests involving only two MGF spikes, we were unable to detect responses in either the anterior or middle regions of the animal.

Fig. 14. Regional variations in MGF-mediated behavioral response. As shown in the upper three traces, light stimulation of the head, sufficient to evoke a train of MGF spikes, resulted in increases in body width in the anterior (A; segments 20-30), middle (M; segments 70-80), and extreme posterior (P; segments 135-145) regions of the animal. The lower three traces show three (at least) MGF spikes (dots) which precede the behavioral response in each of the three regions.

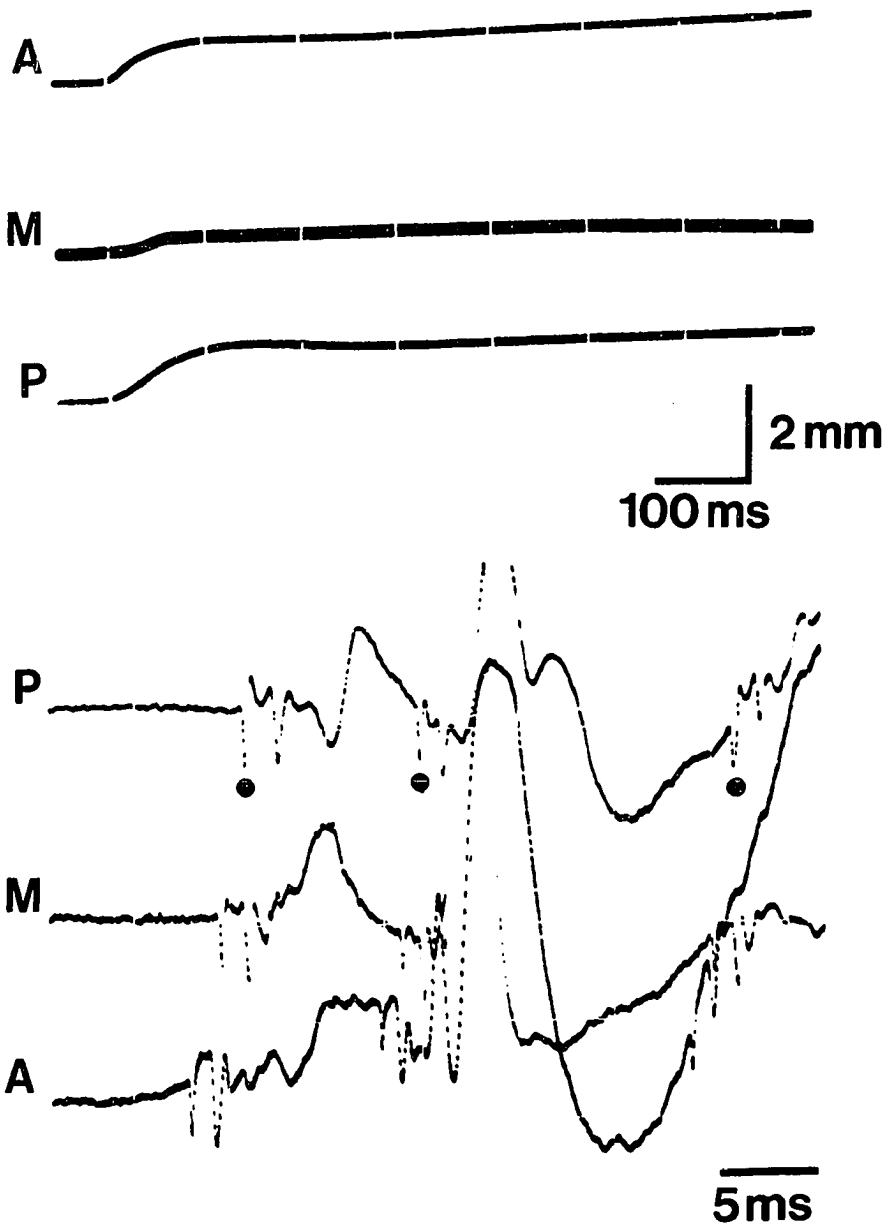

A
M
P
2 mm
100 ms
P
M
A
5 ms

Table 2. MGF-mediated changes in body width in different regions (n = 5 animals)

	Anterior region	Middle region	Tail region
Mean increase in body width (range)	0.8 mm (0.4-1.2)	0.4 mm (0.1-0.8)	2.2 mm (0.8-4.8)
Mean onset latency of response	53 ms (± 14 S.D.)	58 ms (± 22 S.D.)	37 ms (± 7 S.D.)

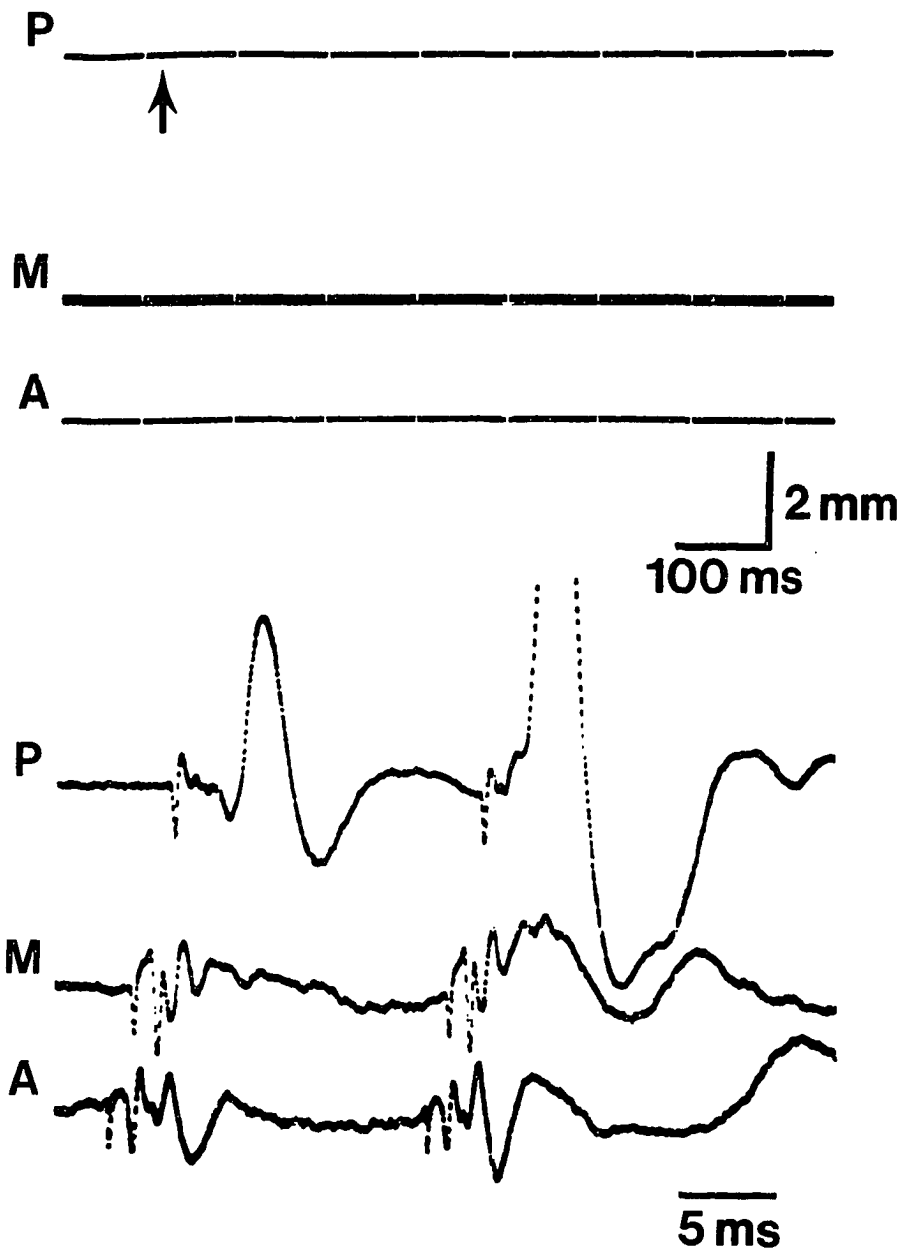
Estimates were made of time lag (onset latency) between arrival of a series of MGF spikes at a particular region and the onset of a detectable behavioral response in that region (Table 2). Onset latency in the middle of the animal was slightly longer than in the anterior.

Recordings were also made of MGF-mediated behavioral responses in the most posterior 20 segments (tail) of the animal (Fig. 14). In this region, the primary behavioral response consists of a large increase in body width accompanied by little or no apparent shortening, but involving a pronounced dorso-ventral flattening of the tail. Increases in body width at the tail were several times greater than at any other region of the animal (Table 2).

The minimum number of MGF spikes required to evoke tail flattening appeared to be less than was required to evoke detectable changes in width in the middle or anterior regions of the body. Figure 15, for example, shows that a very slight flattening of the tail can occur in response to a pair of MGF spikes, even though no responses were detectable in the anterior or middle regions of the animal.

Onset latency for the tail flattening response (Table 2) was considerably shorter than for the responses in other regions of the animal.

Fig. 15. Behavioral (upper three traces) response accompanying two MGF spikes (lower three traces). Light tactile stimulation of the head evoked two MGF spikes (interspike interval approximately 17 ms). A slight tail flattening (arrow) was seen in the extreme posterior (upper P; segments 135-145) region of the animal. Note the facilitating muscle potentials in the corresponding electrophysiological record (lower P) for the same region. No behavioral responses were detected in either the anterior (A; segments 20-30) or middle (M; segments 70-80) regions of the animal.


P
M
A
2 mm
100 ms
P
M
A
5 ms

DISCUSSION

The giant fiber systems mediating rapid escape behavior in a wide variety of segmented invertebrates course nearly the entire length of the central nervous system. In groups in which there is obvious anatomical and physiological specialization of body segments (e.g., arthropods), it is not surprising that there is corresponding anatomical and physiological specialization in the segmental arrangement of the afferent, central and efferent components of the giant fiber reflex. For example, in the cockroach, giant nerve fibers are known to receive sensory input from the abdominal cerci and, when excited, activate antennal muscles and thoracic motor neurons (Dagan and Parnas, 1970; Parnas and Dagan, 1971; Ritzmann and Camhi, 1976).

Perhaps one of the best understood systems, with respect to longitudinal variations in giant fiber reflex pathways is that of the crayfish. Activation of the MGF by visual or rostral tactile stimuli causes simultaneous excitation of the anterior abdominal flexor motor neurons and the homologous flexor motor neurons of the telson-uropod segment, resulting in complete abdominal flexion (Larimer, Eggleston, Masukawa and Kennedy, 1971). The LGF, activated by phasic tactile stimulation of the abdomen (Zucker, 1972a), excites the central abdominal flexor motor neurons and motor neurons innervating the

productor exopodite muscle, causing an incomplete abdominal flexion accompanied by promotion of the uropods. As a consequence of these regional variations in giant fiber reflex pathways and associated behaviors the crayfish can escape by either a posterior and horizontal thrust (MGF response) or a posterior and vertical thrust (LGF response).

The possibility that regional variations exist in the giant fiber reflexes of annelids has been given little attention. Horridge (1959), in studying the rapid escape response of Nereis, reported that the paramedial giant fibers are excited by mechanical stimulation of the posterior end and initiate longitudinal shortening and backward pointing of the parapodia. The dorsal median giant fiber, excited by stimulation of the first 60 segments, produces longitudinal shortening and forward pointing of the parapodia. The lateral giants, excited by stronger stimulation anywhere along the body, apparently also cause longitudinal shortening.

In earthworms previous studies have shown that somewhat comparable regional variations exist with respect to sensory inputs to the giant fibers (Bullock, 1945; Adey, 1951). GÜnther (1973), further clarifying the work of Bullock, showed that the sensory field for the MGF in Lumbricus is most concentrated anteriorly but extends posteriorly well beyond segment 50, overlapping extensively the sensory field of the LGF (approximately the posterior three-fourths of the animal).

Once activated, the MGF conducts very rapidly relative to nonmyelinated invertebrate giant fibers of comparable diameter, a consequence of the myelin-like sheath around the MGF and the fiber's ability to conduct by saltatory conduction (GÜnther, 1976). In this study, we have shown that MGF conduction rate varies along the animal, and that there are corresponding variations in MGF diameter. MGF diameter and conduction rate are greater (Fig. 8) in the anterior one-half of the animal than in posterior regions. The functional significances, if any, of the observed variations are not known. These variations do not appear to modify either the one-to-one nature or temporal aspects of MGF-to-GMN1 coupling along the animal, and, therefore, may be of little consequence in producing regional variations in MGF-mediated escape responses. Rather, behavioral variations appear to derive from regional specializations in the efferent components of the MGF reflex pathway.

Previous studies have shown that repeated firing of MGF and GMN1 spikes is accompanied by facilitating electrical potentials in the longitudinal muscle (GÜnther, 1972; Drewes, Landa and McFall, 1978), but there has been no study of regional variations of either these muscle potentials or the behavior which accompanies them. The regional variations we have observed in facilitation of muscle potentials (Fig. 12) suggest that properties of GMN1 neuromuscular junctions

differ along the length of the animal. In anterior regions the relatively large amount of facilitation may be the result of greater transmitter release or greater sensitivity of the post-synaptic membrane to transmitter relative to central regions of the body.

The large facilitation of muscle potentials seen in the anterior one-third of the animal correlates well with the relatively large increase in body width (longitudinal shortening) recorded in this same region. The shortness of onset latency values for anterior regions (Table 2), relative to middle regions, is consistent with the idea that fewer MGF spikes in a series are required in anterior than in posterior regions to evoke a sufficient level of longitudinal muscle excitation and contraction to produce a measurable increase in body width. Indeed, we have often observed that longitudinal shortening responses to very light tactile stimulation of the head may occur in anterior regions with no measurable behavioral response being seen in the middle of the animal.

The tail flattening which normally accompanies MGF-mediated rapid longitudinal escape contractions has been briefly noted by previous investigators (Bovard, 1918; Rushton, 1945) but prior to this study has never been examined in any detail. As shown in Fig. 14 the onset of this flattening can occur at essentially the same instant that longitudinal shortening begins in the

animal. In fact, considering that the total MGF conduction time along the entire animal is only about 3-5 msec, the onset latency values given in Table 2 would indicate (supported by our unpublished records) that the flattening response often begins several milliseconds before initiation of shortening in the anterior end. This could be due, in part, to the fact that the minimum number of MGF spikes required to evoke tail flattening may sometimes be less than the number required to evoke longitudinal shortening in anterior and middle regions (Fig. 15). Alternatively, regional differences in conduction time along the efferent pathway or in time required for muscle excitation may account for these observations. In any event, considering 1) the close association of MGF spiking with tail flattening, 2) the short time from stimulus onset in the anterior end to the beginning of tail flattening (approximately 50-100 ms), and 3) the absence of any other known sufficiently rapid and through-conducting pathway, we must conclude that the response is MGF-mediated.

The anatomical and physiological basis of tail flattening has not yet been studied. Neither longitudinal nor circular muscle layers appear to be arranged in such a way that they could mediate this response. Preliminary observations in our laboratory suggest that one muscle group which may be involved in tail flattening is the septal musculature. In most body segments the

septa are thin and contain muscle fibers oriented in a variety of directions (Stephenson, 1930). They appear to be involved in the dynamics of peristaltic movements of the gut and body wall (Seymour, 1976).

In the extreme posterior portion of the animal, however, the septa are thickened and the septal musculature is very well developed. Preliminary histological examinations of posterior septa, as well as observation of their movement in dissected preparations, suggest that there are significant numbers of dorso-ventrally oriented muscle fibers whose contraction may contribute to the MGF-mediated tail flattening response. It is important to note, however, that it is not yet clear whether muscle potentials recorded from the extreme posterior end of the animal actually arise from units mediating tail flattening. It is possible that activity in the flattening muscles cannot be detected through the ventral surface of the body wall, in which case recorded muscle potentials would necessarily arise from other muscle elements.

An interesting observation is that immature worms have approximately 15-20 fewer segments than adult worms and the flattening response is not as pronounced. Possibly as new segments are added posteriorly near the anus (Stephenson, 1930) the tail flattening ability may also develop in those segments.

A more subtle aspect of the MGF-mediated escape behavior currently under investigation pertains to regional variations in the actions of the setal muscles. Observations made by Rushton (1945) suggest that rapid longitudinal escape contractions are accompanied by protraction of the setae in the tail. This helps to anchor the animal's tail in the burrow during escape. In more anterior regions it seems reasonable that the opposite action (i.e. setal retraction) would be advantageous during rapid withdrawal toward the burrow.

SUMMARY

1. Methods are described for simultaneously monitoring at multiple sites the electrophysiological and behavioral events associated with giant fiber-mediated escape in intact earthworms.

2. Regional differences in the conduction properties and diameter of the MGF and in properties of GMN1-mediated muscle potentials are reported; properties of MGF-to-GMN1 coupling appear similar throughout the animal.

3. The amount of facilitation of GMN1-mediated muscle potentials increases along a posterior-to-anterior gradient.

4. Generally, facilitation of GMN1-mediated muscle potentials increases as a function of decreasing MGF interspike intervals.

5. Regional differences in the MGF-mediated behavior are described; longitudinal contractions are largest in the anterior regions, consistent with observed patterns of muscle potential facilitation.

6. Dorso-ventral flattening of the tail during escape is characterized and shown to be MGF-mediated.

7. The magnitude of the tail flattening response, as well as longitudinal shortening of the body, are graded depending on the number of MGF spikes and interspike intervals.

PART III. LONGITUDINAL VARIATIONS IN THE PROPERTIES OF THE LGF-GMN2 PATHWAY

INTRODUCTION

The lateral giant fiber system (LGF) of the earthworm consists of two parallel through-conducting fibers, each formed by the tandem arrangement of large segmental interneurons (Mulloney, 1970). In each segment the two LGF's are connected to one another by electrotonic junctions, so the two fibers spike synchronously as though they were a single unit (Wilson, 1961). Such spiking can be evoked by tactile stimulation within the LGF sensory field which includes approximately the posterior three-fourths of the animal (Günther, 1973).

The efferent pathway associated with the LGF in each segment consists of a pair of motor neurons, designated GMN2 (Günther and Walther, 1971; Günther, 1972). Each GMN2 axon divides into two branches which exit the ventral nerve cord contralaterally via the third segmental nerve of the same ganglion and the first segmental nerve of the adjacent posterior ganglion. Spikes in GMN2 axons are accompanied by large extracellular potentials in the longitudinal muscle layer (Günther, 1972). In isolated nerve-muscle preparations the mechanical response accompanying excitation of a GMN2 axon in one of the segmental nerves consists of a very small contraction of the longitudinal muscle (Drewes

and Pax, 1974b). Presumably activation of numerous GMN2's along the animal would result in a pronounced longitudinal shortening (escape response) of the animal. Detailed analysis of such behavioral responses from intact preparations has not been made. Accordingly, we studied LGF-to-GMN2-mediated escape responses in intact animals, giving special attention to regional variations in the central and efferent components of the reflex pathway.

MATERIALS AND METHODS

Methods for monitoring electrophysiological and behavioral responses of intact animals, as well as methods for anatomical study, were identical to those described in the preceding paper. In all cases LGF activity was initiated by tactile stimulation of the most posterior few segments of the animal.

RESULTS

Regional Variations in LGF Conduction Rate and Diameter

Results summarizing regional variations in LGF diameter and conduction rate are presented in Fig. 16. LGF diameter is greatest, approximately 50 μ, in the most posterior quarter of the animal, decreasing uniformly to approximately 35 μ in the most anterior quarter. These values correspond with observed differences in LGF conduction rate along the animal. The grand mean for LGF conduction rate measured in the anterior one-half of nine animals was 10.6 m/s; the grand mean in the posterior one-half was 13.0 m/s. This slowing of conduction rate in the anterior regions can be seen in Fig. 17. Using a paired difference t-test (Mendenhall, 1968) the mean conduction rate in the anterior and posterior halves of the animal were found to be significantly different ($p < 0.001$).

We also tested for regional variations in the ability of the LGF to facilitate its conduction rate. Data were taken from tests in which LGF spike-pairs (interspike intervals $\leq$ 15 ms) were evoked by light tactile stimulation of the tail. The grand mean conduction rate for second spikes in the posterior one-half of the animal was 14.9 m/s ± 0.5 S.E.M. ($n = 8$) representing approximately a 15 percent increase in conduction rate compared to that of first spikes. In the anterior one-half the grand mean was 12.3 m/s ± 0.5 S.E.M. ($n = 8$), approximately a 16 percent increase compared

Fig. 16. Regional variation in LGF diameter and conduction rate. Bars indicate grand mean diameter (vertical lines represent S.E.M.; n = 5 animals) for each quarter of the animal. The grand mean conduction rates (n = 9 animals) are shown for anterior and posterior halves of the animal.

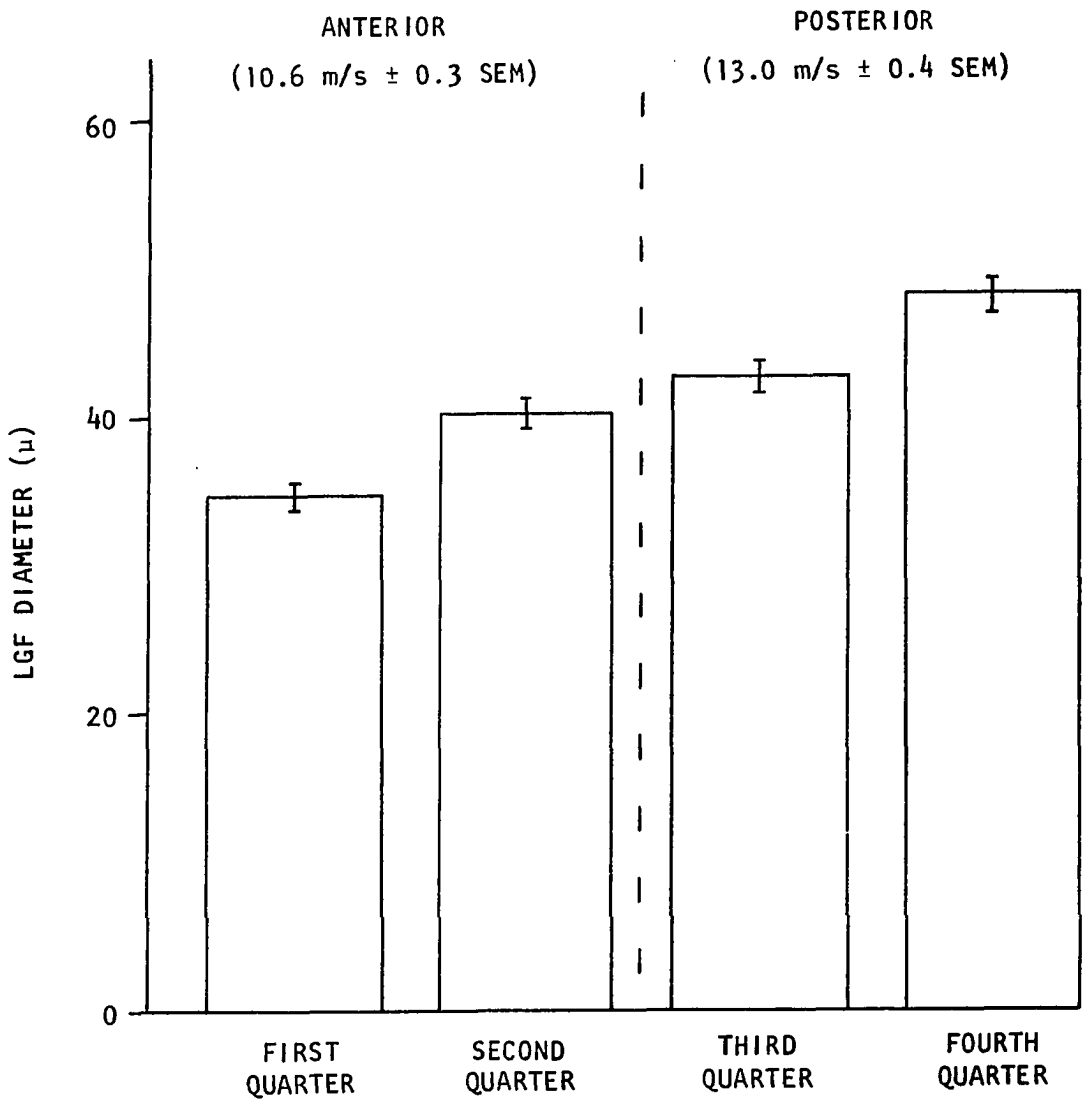
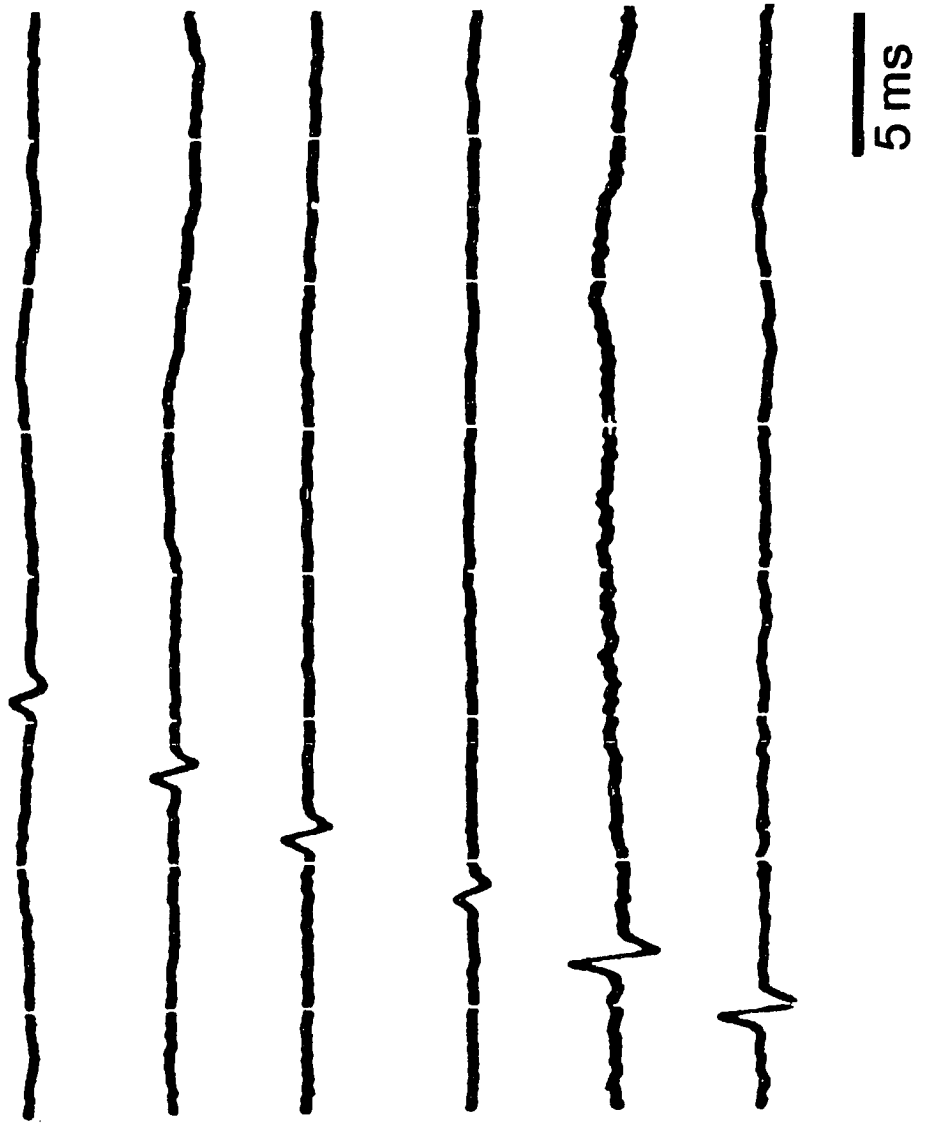

ANTERIOR
(10.6 m/s ± 0.3 SEM)
POSTERIOR
(13.0 m/s ± 0.4 SEM)
LGF DIAMETER (μ)
60
40
20
0
FIRST QUARTER
SECOND QUARTER
THIRD QUARTER
FOURTH QUARTER

Fig. 17. Simultaneous recordings of a single LGF spike from six different sites along the animal. Recording sites were 20 mm apart, the most anterior site (upper trace) at approximately segment 25 and the most posterior site (lower trace) at approximately segment 140. A very light touch of the animal's tail evoked a spike which was conducted in a posterior-to-anterior direction. Note that the conduction rate is slower in anterior regions than in posterior regions of the animal.


5 ms

to the conduction rate of first spikes in the same region. These results suggest that the ability of the LGF to facilitate its conduction rate is similar along the animal.

Regional Variations in LGF-GMN2 Coupling

In studies of dissected preparations GUnther (1972) has shown that the first LGF spike in a series never evokes a GMN2 spike, but that second and subsequent LGF spikes are coupled to GMN2 spikes in a 1:1 fashion. He reported that delay times between LGF and GMN2 spikes were variable (approximately 0.9-3.5 ms). In this study we show that during LGF responses the GMN2 spike potentials and their accompanying large muscle potentials can be recorded from the ventral surface of intact animals (see Figs. 18-20). Delay times between LGF and GMN2 spikes in intact animals were also variable, but always within the range of 1.0-3.2 ms. Delay times between GMN2 spikes and the onset of accompanying muscle potentials ranged from 0.4 to 1.4 ms (mean 0.8 ms; n = 5). Differences between the means of these delays in different regions of the animal were at most a few tenths of a millisecond. The short delay between the GMN2 spike potential and onset of the muscle potential indicates that it may be a reasonable estimate of synaptic delay. If so, then GMN2 spike potentials in our recordings are probably being detected peripherally from the segmental nerve relatively

Fig. 18. Response to two closely spaced LGF spikes. Recording sites were spaced 12 mm apart, the most anterior site (lower trace) at approximately segment 30 and the most posterior site (upper trace) at approximately segment 135. Light tactile stimulation of the tail evoked a pair of LGF spikes (interspike interval approximately 4 ms). At the three most posterior and two most anterior recording sites the second LGF spike was followed by a small GMN2 spike potential (dots) and a very large muscle potential. In the fourth trace (approximately segment 70) no GMN2 spike or large potential was detected.

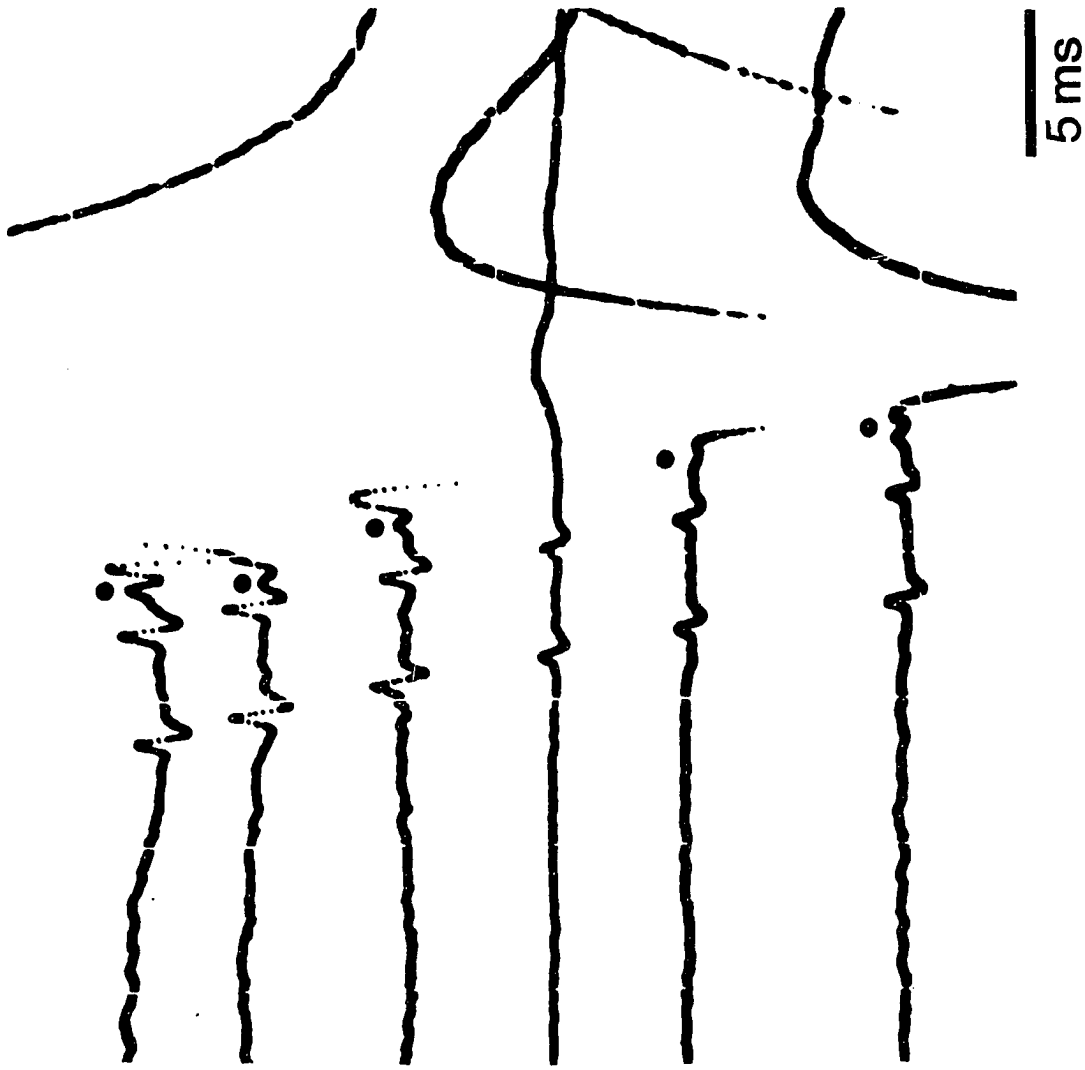

5 ms

Fig. 19. Regional variation in the effectiveness of 2:1 coupling of LGF and GMN2 spikes. Each of four animals, A-D, was tested repeatedly. A test consisted of the occurrence of two LGF spikes (interspike intervals 2-6 ms) at a particular recording site in one of the five regions. Bars represent the percentage of tests in a given region in which successful 2:1 coupling was observed. Total numbers of tests for the four animals are A, n = 86; B, n = 107; C, n = 76; D, n = 116.

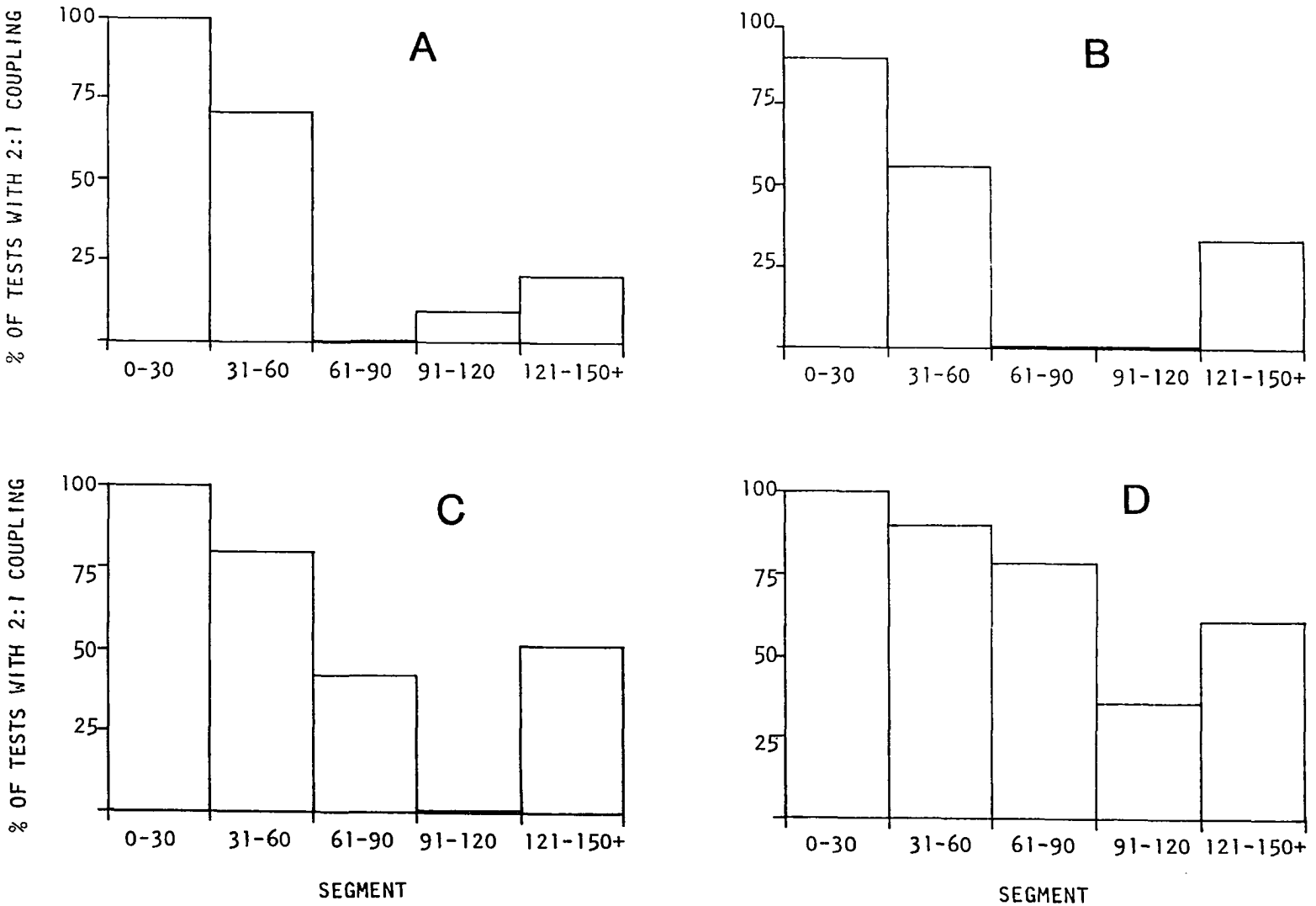
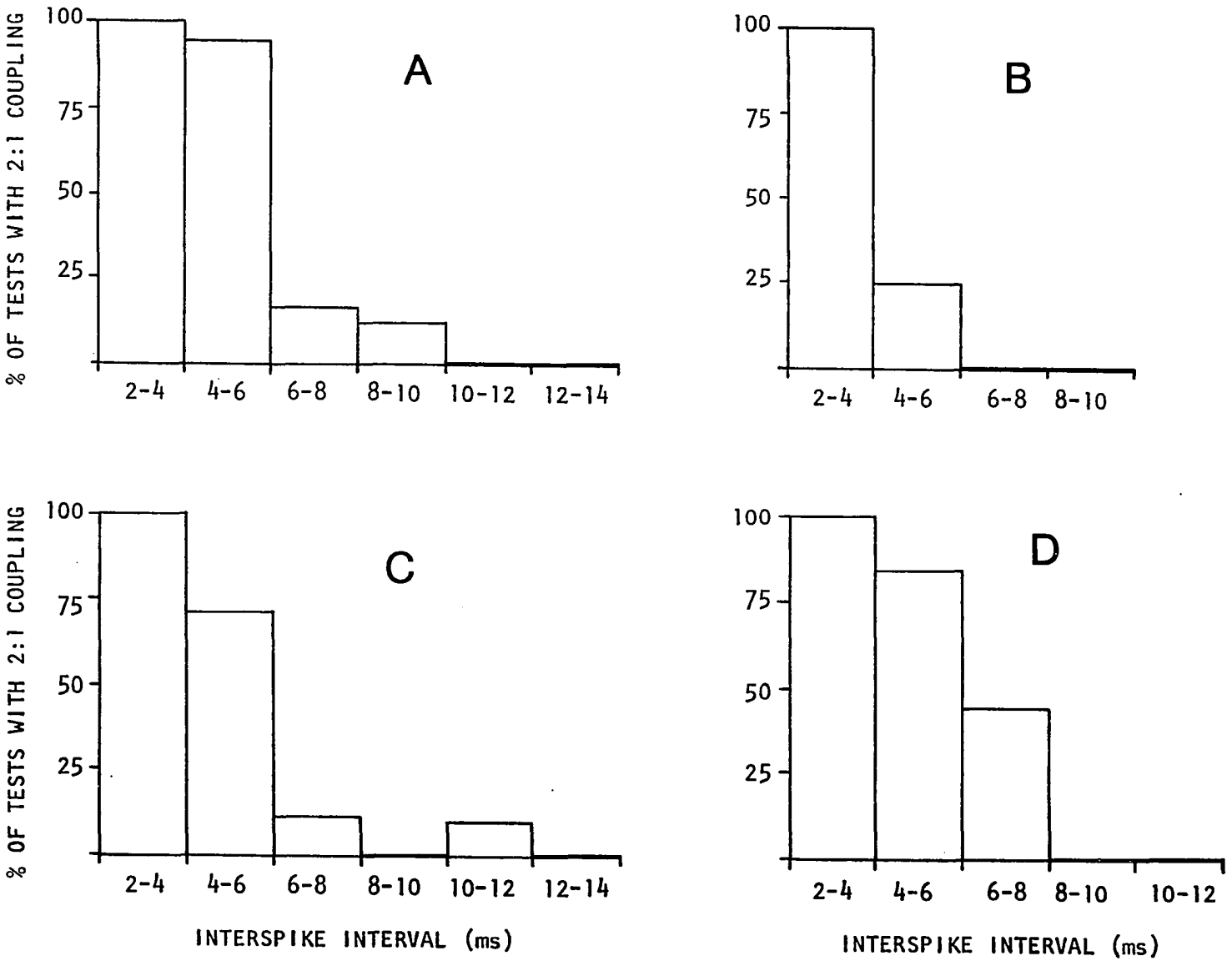

% OF TESTS WITH 2:1 COUPLING
100
75
50
25
A
0-30
31-60
61-90
91-120
121-150+
B
C
D
SEGMENT

Fig. 20. Effect of LGF interspike interval on successful LGF-GMN2 coupling. Each of four animals, A-D, was tested repeatedly by evoking pairs of LGF spikes with different interspike intervals. Bars indicate the percentage of tests in a given interval range in which two LGF spikes were successfully coupled to a GMN2 spike. Total numbers of tests for each of the four animals are A, n = 51; B, n = 72; C, n = 64; D, n = 43. All data were taken from the region of segments 31-60.


% OF TESTS WITH 2:1 COUPLING
100
75
50
25
A
2-4
4-6
6-8
8-10
10-12
12-14
B
2-4
4-6
6-8
8-10
C
2-4
4-6
6-8
8-10
10-12
12-14
INTERSPIKE INTERVAL (ms)
D
2-4
4-6
6-8
8-10
10-12
INTERSPIKE INTERVAL (ms)

near the synaptic connections of the GMN2 axon with longitudinal muscle.

A particularly striking feature of the LGF-GMN2 pathway in intact animals was regional variation in the effectiveness of 2:1 coupling between the LGF and GMN2. These variations in coupling effectiveness were examined by determining the percentage of tests in which two LGF spikes evoked a single GMN2 spike (Figs. 18-19). In all animals this 2:1 coupling was most effective in anterior regions, being nearly 100 percent effective in the first 30 segments. In two animals showing particularly effective coupling in this region single LGF spikes occasionally evoked GMN2 spikes. Although somewhat variable in different animals, the effectiveness of 2:1 coupling always diminished in the middle body regions. For example, in two animals (Fig. 19, A and B) a pair of closely spaced LGF spikes always failed to evoke a GMN2 spike in segments 61-90. In this region GMN2 spikes were usually seen only after the third or fourth LGF spike in a train. In segments 91-120 LGF-GMN2 2:1 coupling was also weak (range 0-36 percent, n = 4 animals), but in segments 121-150+ (extreme posterior region) 2:1 coupling was much stronger (range 20-60 percent, n = 4 animals). Thus the greatest tendency for 2:1 coupling of LGF to GMN2 spikes occurs in the anterior and extreme posterior regions of the animal.

The effectiveness of LGF-GMN2 2:1 coupling, in addition to

being dependent on location along the body, is also dependent on LGF interspike interval. Figure 20 shows the relationship between 2:1 coupling (LGF-GMN2) and LGF interspike interval in segments 31-60 of four animals. When LGF interspike intervals were 6 ms or less, 2:1 coupling was highly effective in most animals (e.g., Fig. 20, A, B, and C), but with intervals longer than 10 ms coupling was seldom seen. In other regions of the animal, inverse relationships between LGF-GMN2 coupling success and LGF interspike interval were also observed.

Regional Variations in LGF-Mediated Behavior

A very light tactile stimulation of the animal's tail often evokes a pair of closely spaced LGF spikes. Due to regional differences in the effectiveness of LGF-GMN2 coupling, GMN2 spikes and their associated muscle potentials occur preferentially in the anterior regions of the animal (Fig. 21). In most animals such activity was accompanied by a barely detectable increase in body width (shortening) in anterior, but not middle or posterior regions.

With stronger tactile stimulation trains of LGF spikes evoked GMN2 spikes and their associated large muscle potentials in all regions of the body. As shown in Fig. 22, the large muscle potentials can occur in a repetitive fashion suggesting repeated firing of GMN2, probably in 1:1 correspondence with LGF spikes (GUnther, 1972). Such activity was accompanied by increases

Fig. 21. Response to two closely spaced LGF spikes. The upper three traces show changes in body width in the anterior (A, approximately segments 20-30), middle (M, approximately segments 70-80), and posterior (P, approximately segments 120-130) regions in response to a pair of LGF spikes evoked by light tactile stimulation of the tail. Except in the anterior region (lower A), the two LGF spikes failed to evoke a GMN2 spike. Accordingly, no changes in body width (shortening) were detected in either the middle or posterior regions; however, a slight increase in width occurred in the anterior region. Note that the LGF interspike interval is more than 1 ms longer in the posterior than in the anterior region, a consequence of facilitation of conduction velocity for the second LGF spike.

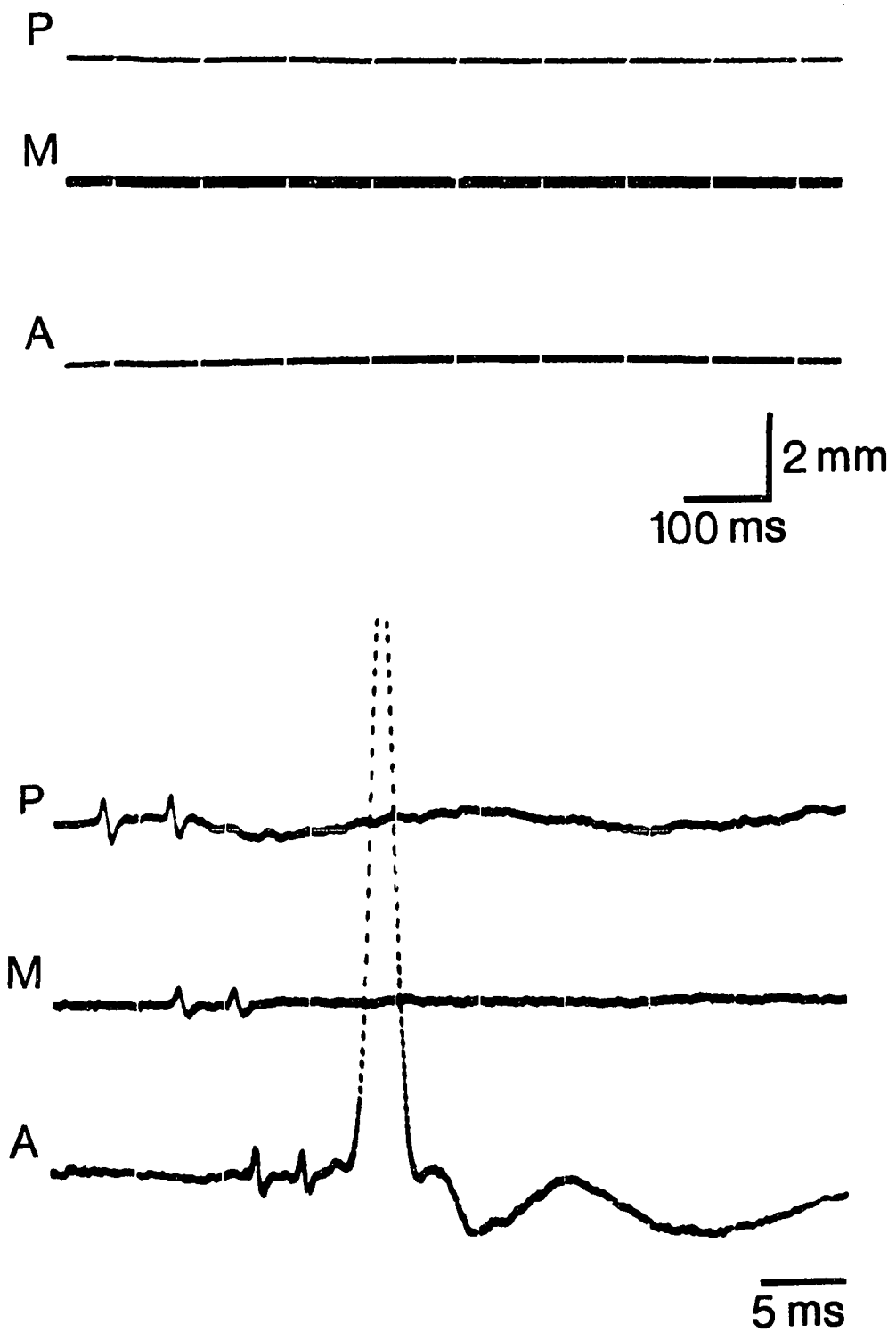
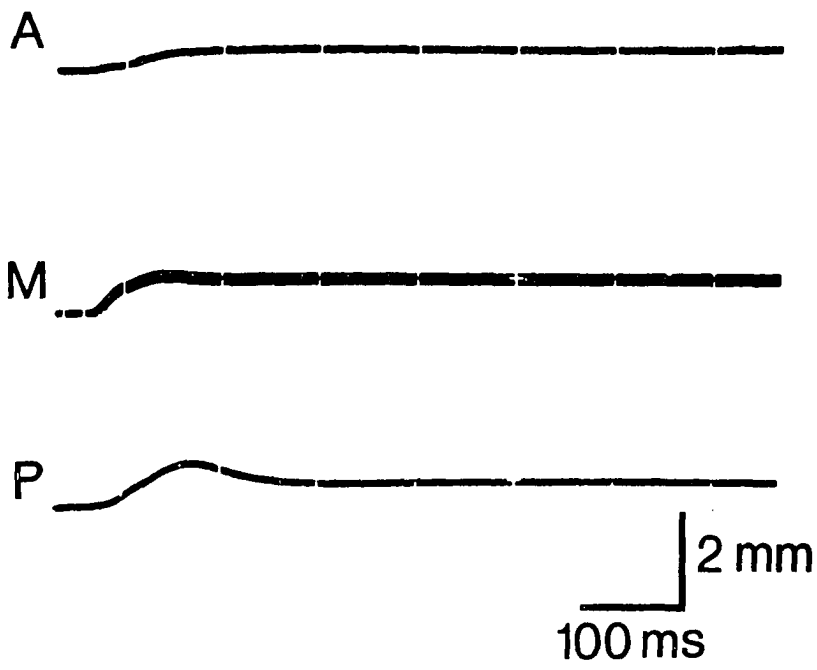
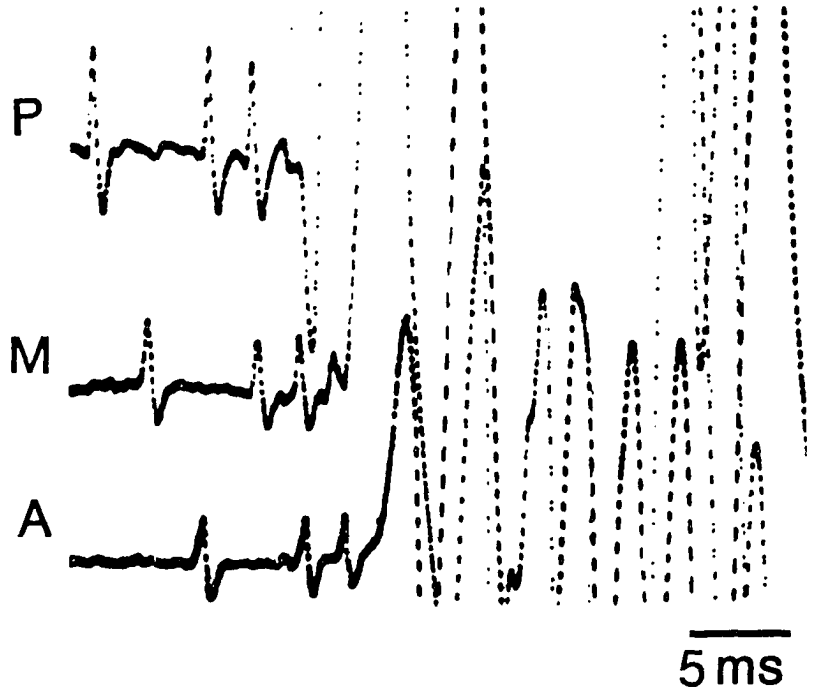

P
M
A
2 mm
100 ms
P
M
A
5 ms

Fig. 22. Regional variation in LGF-mediated behavioral response to moderate tactile stimulation of the tail. The upper three traces show increases in the anterior (A, approximately segments 20-30), middle (M, approximately segments 70-80), and posterior (P, approximately segments 120-130) regions of the animal. Lower three traces show repeated LGF spiking recorded from the same regions. GMN2 activity, as indicated by the small spike potential and large muscle potentials, occurred after the third LGF spike. A relatively sustained increase in body width (longitudinal shortening) was seen in all regions.


A
M
P
2 mm
100 ms


P
M
A
5 ms

in body width (shortening) in all regions of the animal. These behavioral responses were graded in amplitude, depending on the strength of stimulation and the number of presumedly GMN2-mediated muscle potentials.

Table 3 indicates that there are slight regional variations in the mean increases in body width seen during responses similar to those shown in Fig. 22. Generally, responses to moderate levels of stimulation were greater in the tail than in the middle or anterior regions. This contrasts with responses to very light tactile stimulation (Fig. 21).

Table 3. LGF-mediated changes in body width (shortening) in different regions. (n = 5 animals)

	Anterior region	Middle region	Tail region
Mean increase in body width (range)	0.8 mm (0.5-1.4)	0.8 mm (0.7-1.0)	1.4 mm (1.0-1.6)
Mean onset latency of response	42 ms (± 5 S.D.)	40 ms (± 4 S.D.)	41 ms (± 5 S.D.)

DISCUSSION

Evidence from the present and previous studies indicates that regional variations exist in the sensory, central, and efferent components of the earthworm giant fiber reflex. As shown in previous studies (Rushton, 1945; Günther, 1973), the sensory field of the LGF system extends throughout approximately the posterior two-thirds of the animal's body, but is most concentrated at the tip of the tail. This sensory distribution would allow the animal to detect direct and threatening tactile stimulation along most of the body, but in addition would allow the tail, due to its greater sensitivity, to detect slight substrate vibrations occurring while the animal lies extended from its burrow at night.

Results of the present study indicate that there are also regional variations in the central pathway of the LGF escape reflex. LGF diameter and conduction rate decrease along a posterior-to-anterior gradient, but it is not yet known if these differences are in any way related to regional variations in sensory or efferent pathways involved in escape.

In addition to the differences in LGF diameter, there are also regional variations in the effectiveness of coupling between LGF and GMN2 spikes. This contrasts with the MGF system (previous paper) in which MGF-GMN1 coupling was consistently 1:1 throughout the length of the animal. In the LGF system a single LGF spike

can occasionally evoke a single GMN2 spike, but only in anterior segments. Usually a pair of closely spaced LGF spikes is required to evoke a GMN2 spike in anterior segments and as shown in Fig. 19 the probability of this 2:1 coupling is substantially reduced in more posterior segments (segments 61-120). In addition to these regional influences the probability of successful coupling is increased by reduction in LGF interspike interval (Fig. 20). The ability of the LGF to facilitate its conduction rate would thus tend to accentuate regional differences in coupling by reducing the LGF interspike interval in anterior regions. For example, a 15-20 percent facilitation of conduction rate for the second of a pair of LGF spikes in a 15 cm long worm would reduce the LGF interspike interval by approximately 1-2 ms (Fig. 21). Thus the probability of LGF-GMN2 coupling near the head could be substantially increased (Fig. 20).

The significance of highly effective LGF-GMN2 coupling in the anterior regions may be related to the rapid escape behavior commonly observed at night when the animal's body is extended from its burrow. Detection of substrate vibrations by the tail in the burrow may lead to a firing of one or a few LGF spikes. This level of spiking could produce a GMN2-mediated rapid withdrawal or shortening in the anterior regions. Since there exists a second zone of enhanced LGF-GMN2 coupling in the extreme posterior region (approximately segments 121-150+), a shortening

and increased diameter of the tail could occur, possibly aiding in anchoring the animal in the burrow as occurs in the MGF response. Under laboratory conditions these levels of LGF spiking can easily be evoked by slight vibration of the experimental apparatus. Such spiking, however, is usually accompanied only by very slight longitudinal contractions. This may indicate a long-term central or peripheral depression in the GMN2-mediated behavioral responses. Field studies now in progress should determine whether there are differences between LGF-mediated escape responses in the laboratory and field, and whether there are functional parallels between LGF-mediated and MGF-mediated escape responses.

In contrast to the anteriorly focused motor responses which accompany only a few LGF spikes, motor responses evoked by stronger tactile stimulation and longer trains of LGF spikes are distributed throughout the length of the body (Fig. 22). This widespread and pronounced shortening response to strong, direct stimulation of the body may be of behavioral significance as a generalized protective withdrawal which may occur during many different types of activity.

SUMMARY

1. Regional variations in the diameter of the earthworm LGF correlate well with differences in LGF conduction rate, both being greatest (44-49 μ and 13.0 m/s) in the posterior region and less (34-40 μ and 10.6 m/s) in the anterior regions.

2. Recordings from the ventral surface of intact animals indicate that giant motor neuron (GMN2) spikes and their associated muscle potentials can be evoked in response to LGF spiking.

3. In the first 60 and last 30 segments of the animal LGF-GMN2 coupling is particularly effective, two closely spaced LGF spikes usually evoking a single GMN2 spike; 2:1 coupling is least effective in the middle region of the animal (segments 61-120).

4. In all body regions, the effectiveness of LGF-GMN2 coupling is inversely related to LGF interspike interval; thus the highly effective LGF-GMN2 coupling in anterior regions is enhanced by facilitation of LGF conduction rate which tends to reduce LGF interspike interval.

5. Successful coupling of one or two LGF spikes to GMN2 spikes preferentially in the anterior and extreme posterior regions may be advantageous by localizing the rapid withdrawal behavior in an animal extended from its burrow.

PART IV. A MICROCOMPUTER-CONTROLLED ELECTRONIC SWITCHING SYSTEM FOR MULTICHANNEL ELECTROPHYSIOLOGICAL RECORDING

INTRODUCTION

An important approach to understanding neural functioning and behavior has been the use of multiple channel electrophysiological recording techniques which permit monitoring of bioelectrical activity at numerous selected sites on animals. For example, EKG, EEG and EMG activity can be easily recorded using multiple electrodes attached to surface or subsurface locations (Thompson and Patterson, 1974). Also printed circuit microelectrodes have recently been described (Pickard and Welberry, 1976) which now extend multichannel microelectrode recording capabilities to as many as 50 separate channels.

Use of multichannel recording systems creates problems related to data acquisition, storage, display and analysis. If the experimenter opts for continuous monitoring of all available signal sources he must have suitable instrumentation for multichannel display and storage of data, but may then be faced with the problem of data overload. More often, the experimenter intermittently samples selected sources via some type of switching system. A variety of mechanical or electromechanical switching devices have been used (Drewes, Landa, and McFall, 1978; Milligan, 1977), but problems encountered include slow switching speed and substantial

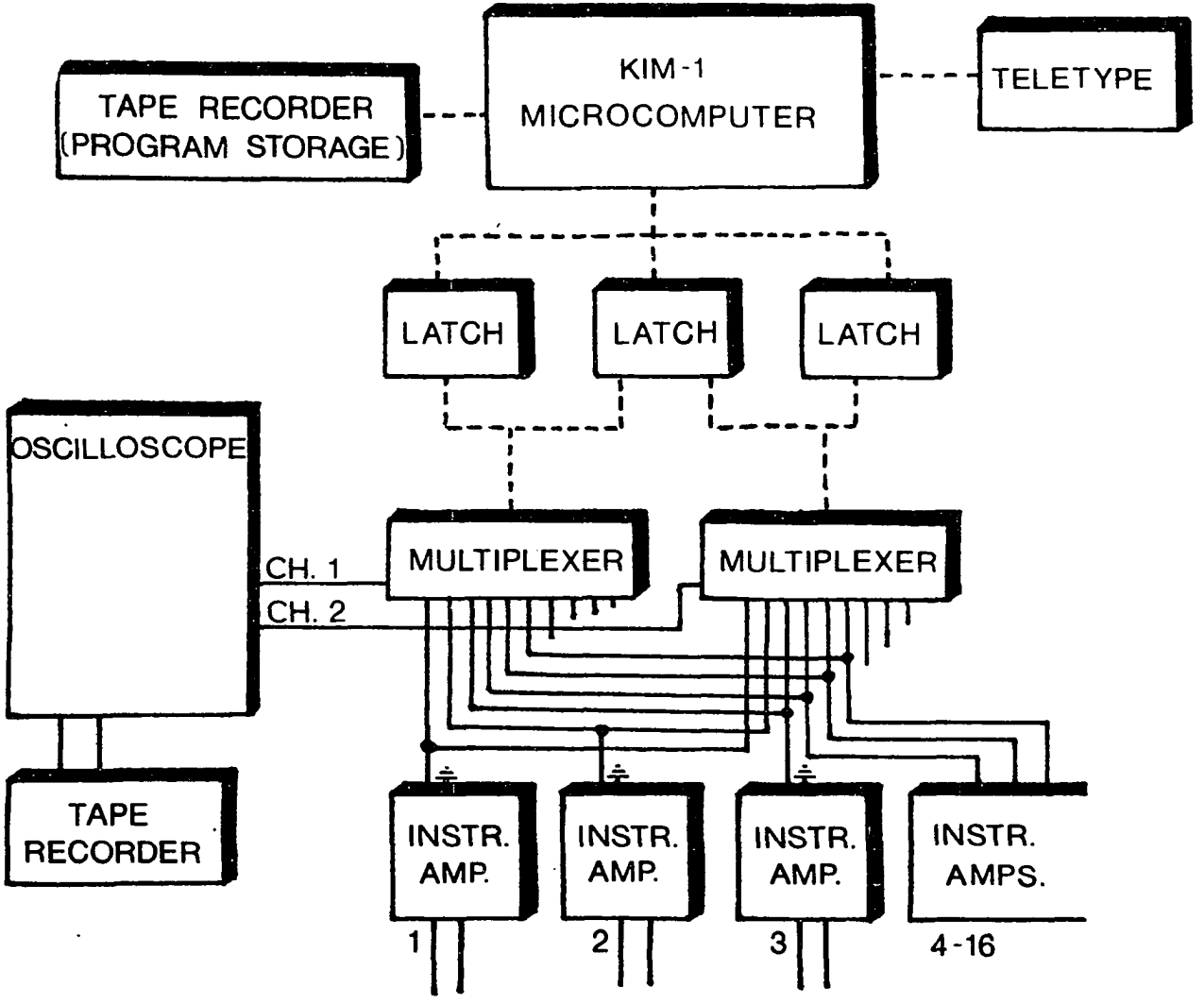
electrical artifacts. We have developed a system which significantly reduces these problems. The system has been successfully applied to multichannel electrophysiological recording of bioelectric activity from intact, freely moving earthworms.

METHODS

Our recording apparatus consists of a circular test arena (Drewes, Landa, and McFall, 1978) plus an electronic switching and amplification system under control of a microcomputer. Earthworms placed in the arena crawl over multiple pairs of silver wire electrodes. Electrical activity from discrete neural units, such as giant nerve fibers, and muscles can then be monitored through the moist skin on the worm's ventral surface. Signals from the electrodes are applied to differential inputs of a bank of integrated circuit instrumentation amplifiers (AD521J, Analog Devices, Norwood, Mass.) (Fig. 1). Amplifier single-ended outputs are applied in parallel to the inputs of two CMOS 16 channel analog multiplexers (Analog Devices AD7506) comprising a two-channel switching system with up to 16 inputs (all inputs available on both channels) or 32 inputs (each input available on only a single channel). The multiplexer outputs are connected to storage oscilloscopes, tape recorders, and other imaging or storage devices.

The experimenter directs switching of recording electrodes as the animal moves around the arena. Switch select signals are generated by the KIM-1 microcomputer (MOS Technology, Inc., Norristown, Pa.) in response to experimenter commands typed on a KSR 33 teletype which also serves as the system printer (Fig. 1). To free the microcomputer for uses other than switching, switch select signals are temporarily stored in three TTL type 7475

Fig. 23. Microcomputer-controlled electronic switching and recording system. Dotted lines indicate switching control circuits. Solid lines indicate recording pathways. Electronic switching and amplifier circuits were easily constructed on hobbyist printed circuit boards.


KIM-1
MICROCOMPUTER
TAPE RECORDER
(PROGRAM STORAGE)
TELETYPE
LATCH
LATCH
LATCH
OSCILLOSCOPE
CH. 1
CH. 2
MULTIPLEXER
MULTIPLEXER
TAPE
RECORDER
INSTR.
AMP.
INSTR.
AMP.
INSTR.
AMP.
INSTR.
AMPS.
1
2
3
4-16
INPUTS FROM RECORDING ELECTRODES

latches which provide control levels to the multiplexers (Fig. 2). An ordinary cassette tape recorder, interfaced directly with the microcomputer, provides storage for operating programs.

Switching programs (Flow chart, Fig. 3) were easily written in machine language since teletype interface subroutines were incorporated by the manufacturer into the KIM-1 operating software (stored in read-only memory).

Fig. 24. Details of pin connections for switching system. A_0-A_3 = switch-select lines; EN = chip enable line; C_1 and C_2 = clock lines for channel selection. Each TTL type 7475 latch output requires a 1K pull-up resistor (not shown) connected to + 5 V supply line to provide sufficient drive for CMOS multiplexers (MUX). Pins 4-11 and 19-26 on the multiplexers receive inputs from instrumentation amplifiers. Wiring and supply voltage requirements are from the manufacturers' brochures received with the individual devices.

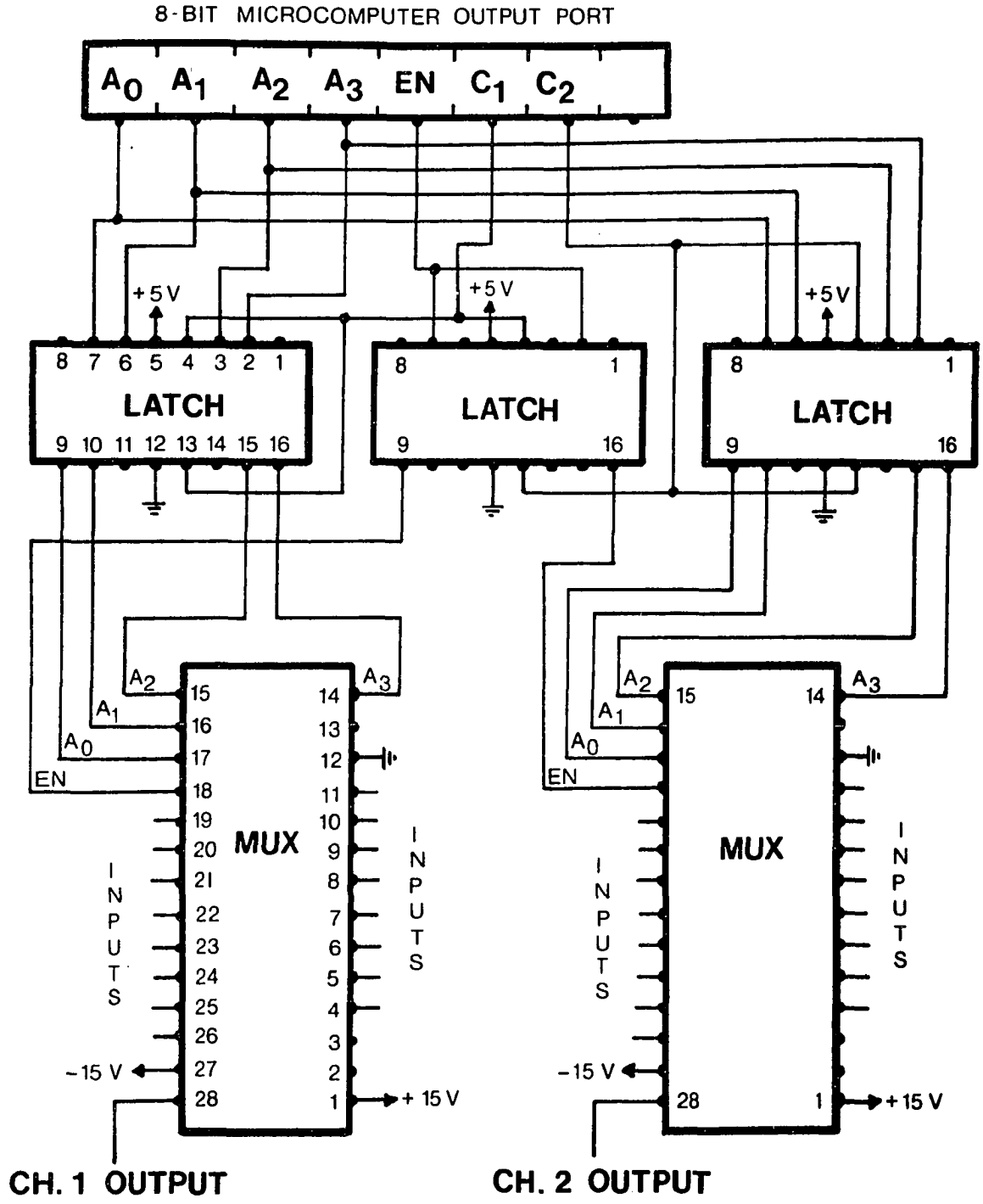
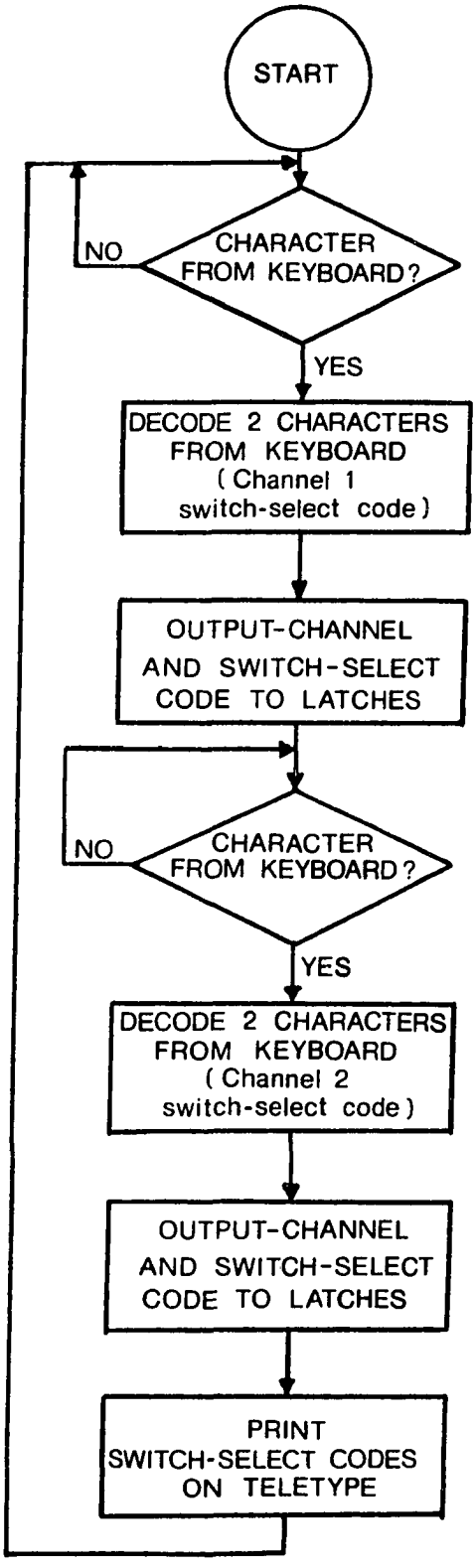

8-BIT MICROCOMPUTER OUTPUT PORT
A0 A1 A2 A3 EN C1 C2
+5V
+5V
+5V
8 7 6 5 4 3 2 1
LATCH
9 10 11 12 13 14 15 16
8 1
LATCH
9 16
8 1
LATCH
9 16
A2
A1
A0
EN
15
16
17
18
19
20
21
22
23
24
25
26
27
28
MUX
14
13
12
11
10
9
8
7
6
5
4
3
2
1
A3
INPUTS
-15 V
+15 V
CH. 1 OUTPUT
CH. 2 OUTPUT

Fig. 25. Program flow chart for channel and switch selection.


START
NO
CHARACTER FROM KEYBOARD?
YES
DECODE 2 CHARACTERS FROM KEYBOARD (Channel 1 switch-select code)
OUTPUT-CHANNEL AND SWITCH-SELECT CODE TO LATCHES
NO
CHARACTER FROM KEYBOARD?
YES
DECODE 2 CHARACTERS FROM KEYBOARD (Channel 2 switch-select code)
OUTPUT-CHANNEL AND SWITCH-SELECT CODE TO LATCHES
PRINT SWITCH-SELECT CODES ON TELETYPE

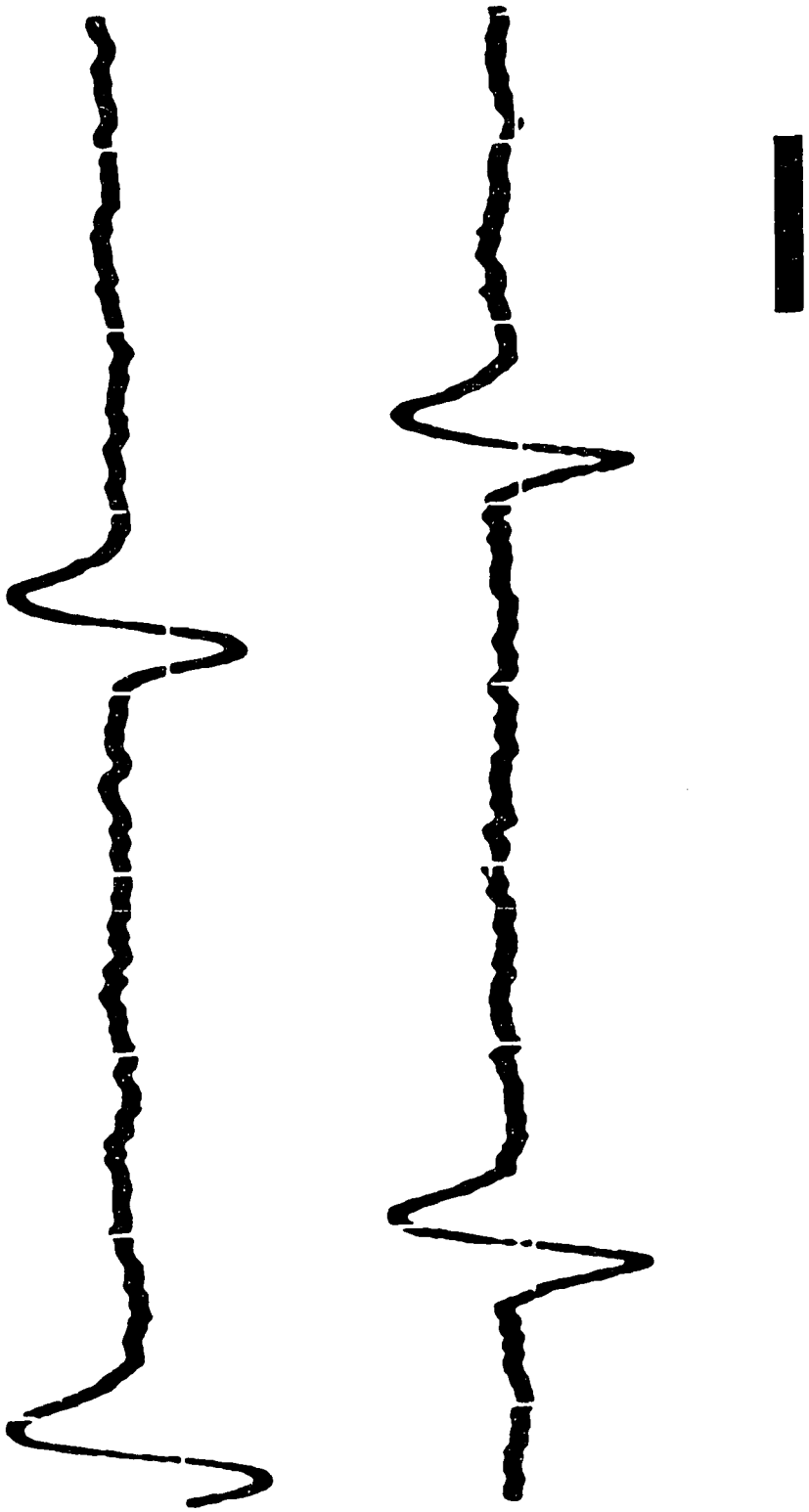
RESULTS AND DISCUSSION

Spike parameters and signal pathway characteristics (Fig. 4) obtained with the electronic switching system are comparable in all respects to those obtained with a mechanical switching system and conventional bioamplifiers (Drewes, Landa, and McFall, 1978). Important differences are the reduction in switching time and elimination of contact artifacts. Switching time in the AD7506 multiplexer is approximately 2 ns, producing artifacts of less than 0.1 s duration in the amplified and displayed signal. This contrasts with the mechanical system in which artifacts caused amplifiers to overload for several seconds or more.

An additional advantage of a microcomputer-based electronic switching system is the ease with which it can be modified to achieve a broad range of experimental objectives. We are currently adding to the system necessary apparatus for noninvasive electrophysiological recording from earthworms in the field. Expanded capabilities will include remote (indoors), simultaneous monitoring and recording from several animals while they enter, exit, or lie extended from their burrows (outside).

Plans for further development of the system include incorporating a computer-controlled digital video system for automated acquisition and analysis of behavioral and electrophysiological data. The system will be particularly suited to longitudinal studies of development, regeneration, and daily/seasonal variations in neural activity and behavior.

Fig. 26. Lateral giant fiber spikes recorded from intact earthworms. Two selected recording sites were located near the middle of the animal, the more posterior site (upper trace) being 20 mm away from the more anterior site (lower trace). A pair of lateral giant fiber spikes was evoked by a light tactile stimulus applied to the posterior end of the animal. The spikes were conducted in a posterior-to-anterior direction at a conduction rate of approximately 10 m/s. The amplitude of individual spikes was typically 50-200μV (SNR 5:1 to 10:1), with low frequency and high frequency filter settings on the CRO amplifiers (Tektronix 5A22N, Beaverton, Ore.) at 100 Hz and 3KHz, respectively. Time scale = 2 ms.



Although we have used no other signal source than our recording arena, the switching system should be applicable to other electrophysiological studies, such as those involving recording from multiple sites on single animals, or from single sites on several animals. Since analytic and sampling routines can be performed under the control of the microcomputer their precision and flexibility are limited only by the expertise of the experimenter-programmer and the amount of computer memory available.

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